STUDIES OF GENERAL AND SEXUAL DEVELOPMENT IN VOLES (*MICROTUS*)

By

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This work is dedicated in remembrance of my father, Raymond John Salo, who conducted comparative research on two species of rabbits at the University of Massachusetts.

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TABLE OF CONTENTS

Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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Three experiments were conducted to investigate how patterns of general and sexual development might be correlated with the formation of social and mating systems among four species of voles (Microtus). Species included pine voles (Microtus pinetorum), prairie voles (M. ochrogaster), meadow voles (M. pennsylvanicus), and montane voles (M. montanus). In Experiment 1, general and sexual development were monitored as voles were exposed to pheromones contained in the soiled bedding from family groups, adult males, or adult females. Few significant effects were found to be due to the treatment. Male pine voles exposed to family or male bedding were significantly heavier than those exposed to clean or female bedding. The uteri of female montane voles exposed to clean or male bedding were heavier than the uteri of those exposed to family or female bedding.

In Experiment 2, the olfactory preferences of voles were measured when they were exposed to male and female bedding on weeks 4, 7, and 10 after birth. Few preferences

were shown for either bedding type by any of the species. Female prairie voles and meadow voles revealed a significant preference for male versus female bedding. Both sexes of all species differed little in the total duration they remained near the female stimulus. Male meadow voles remained near the female stimulus significantly less than the males of the other species on week 10. Female montane voles remained near the male stimulus significantly longer on weeks 4 and 7 than did females of the other species.

In Experiment 3, the influence of the fathers' presence and absence was studied during the rearing of the breeding pairs' first two litters. Pine voles produced their second litter considerably earlier if the male had been present during the rearing of the first litter rather than being absent. Pine voles weaned heavier offspring in the second litter than in the first, when the male had been present for the rearing of the first litter. Montane voles produced litters that were male-biased in sex ratio across both litters, if the male had been present during the rearing of the first litter.

Results are discussed and interpreted from known differences in their contrasting social and mating systems.

CHAPTER 1
GENERAL INTRODUCTION

Statement of the Problem

Several theories have been advanced within the last two decades to account for the evolution of different social and mating systems among birds and mammals (e.g., Emlen & Oring, 1977; Kleiman, 1977; Orians, 1969; Vehrencamp & Bradbury, 1984; Wittenberger 1979). However, not all of the selective forces that underlie the evolution of different mating systems have been identified (see Vehrencamp & Bradbury, 1984). Wittenberger (1979) proposed that because mating behavior is affected by nearly all aspects of an organism's behavioral adjustment to its environment, a theory of mating systems must be integrated with several other types of behavioral theories. For example, theoretical advances concerning the evolution of territoriality, parental behavior, and sociality must be meshed within a broader theory explaining the evolution of mating systems.

One area of study that appears promising for providing greater insights into the evolution of social and mating systems in mammals is research on the regulation of sexual maturation or puberty modulation. For example, although its function remains obscure, delayed sexual maturation is often present among mammalian species that are considered to be monogamous (Kleiman, 1977; Dewsbury, 1981). Some have

suggested that delayed sexual maturation functions to inhibit incestuous mating (McGuire & Getz, 1981), although others have suggested it reduces susceptibility to predation (Batzli et al. 1977). Investigating the causes and function of changes in sexual maturation may lead to an increased understanding of the evolution of different social and mating systems.

A second area of investigation that may offer insight into the formation of the different mating systems is the evolution of male parental care. Paternal care is found most often among mammalian species that form monogamous mating systems and have relatively few offspring (Kleiman, 1977; Dewsbury, 1981). Although it is often assumed that paternal behavior has a beneficial effect on the development of young, the assumption needs to be validated (Wuensch, 1985). The results of several studies conducted to investigate this hypothesis among species of rodents are equivocal (Dewsbury, 1988). It is possible that there are important links between sexual suppression and the evolution of paternal behavior, because the two often occur jointly in monogamous mating systems (Kleiman, 1977; Dewsbury, 1981).

Until recently, the study of developmental processes has been largely divorced from studies of the evolution of mating systems. Muller (1990) noted that ontogeny has been treated as a type of "black box" in evolutionary theory. Similarly, Stearns (1989) discussing developmental processes, noted that the types and sources of phenotypic

variation have been given little consideration in evolutionary theory. The following set of experiments was designed to investigate possible causal links between puberty modulation and paternal care and the resulting differences in social and mating systems in mammals.

Species of the genus Microtus are ideal for studying the functions of sexual maturation and paternal behavior for three primary reasons. First, the species display differences in social and mating system that appear to be associated with puberty modulation and paternal care. For example, the mating systems among Microtus range from monogamy in prairie voles (M. ochrogaster) to promiscuity in meadow voles (M. pennsylvanicus)(Wolff, 1985). Pine voles (M. pinetorum) and prairie voles are considered to be monogamous and appear to be sensitive to the actions of pheromonal cues that affect sexual maturation (Getz & Hofmann, 1986; FitzGerald & Madison, 1983; Carter & Getz, 1985).

A second reason for studying these processes in Microtus is that by investigating differences in puberty modulation and paternal behavior with closely related species, we are most likely to identify the selective pressures and mechanisms that have shaped these species differences (Clutton-Brock & Harvey, 1984; King, 1970; Dewsbury, 1990). A third reason is that many species of Microtus can be bred and maintained within the laboratory where it is possible to systematically vary and control

exposure to stimuli. Such identification and control of stimuli is difficult or impossible with these species under natural conditions. The control offered in the laboratory appears to be a prerequisite to identify the necessary and sufficient stimuli that influence sexual maturation among species. Finally, although there is a considerable literature on such topics as the taxonomy, zoogeography, anatomy, and habitats, of various species of Microtus, currently little is known about many developmental processes such as prenatal development, causes of interspecific variation in litter size, and causes of mortality (Tamarin, 1985; Nadeau, 1985). It is possible that through the systematic investigation of such phenomena, relationships will become evident between them and the selective pressures within the environment which shaped them.

Plan of the Dissertation

Three studies are presented that were designed to investigate the role of two developmental phenomena that might have influenced the evolution and expression of the different social and mating systems among four species of voles (Microtus). The two developmental phenomena include the changes in the timing of puberty (puberty modulation) and influence of paternal presence or absence upon developing offspring. Specifically, Experiment 1 was designed to explore how exposure to naturally occurring pheromones, contained within soiled bedding, influences the timing of puberty in four species of voles. The species

included pine voles (M. pinetorum), prairie voles, (M. ochrogaster), meadow voles (M. pennsylvanicus), and montane voles (M. montanus). In Experiment 2, the behavioral reactions of the four species were studied when they were exposed to soiled bedding in an odor preference task. The study was designed to determine if behavioral preferences for male and female soiled bedding were evident within each species and sex and whether the preferences changed as a function of age. In Experiment 3, the effect of an adult male's presence or absence on his developing offspring was studied. Together the three experiments were designed to explore how these phenomena were related to development and how they might be functionally linked to the expression of different social and mating systems.

Below I present some definitional considerations, present a review of the literature on puberty modulation in house mice (Mus musculus) and voles (Microtus), present a review of the social biology of Microtus, and present the results of the studies designed to investigate puberty modulation and the effects of paternal presence in Microtus. Finally, I attempt to synthesize the results of the three studies with results from other studies to explain possible selective pressures that might have formed and may maintain the different social and mating systems among these species.

Definitional Considerations

There is no globally accepted definition of puberty. According to Hasler (1975), puberty has usually been defined

as the age that animals produce viable gametes. However, in practice, it is often difficult to define the limits of puberty with precision (Bronson & Rissman, 1986). Common measures used in studies with female house mice have included the day of vaginal opening or the first day of an estrous vaginal smear (Drickamer, 1986). It has proven difficult to find a reliable and non-terminal marker to assess puberty in male mice. The presence of viable spermatozoa or the ability to induce pregnancy have been used in some studies as an index of puberty among males (e.g., Vandenbergh, 1971).

Despite debate over how one defines puberty or what one uses as an index of puberty, by chosing a standard measure it is possible to compare differences in the onset of "puberty" as being either accelerated or delayed compared with some reference group. Thus, the term "puberty modulation" is useful when referring to the onset of puberty as being either accelerated, delayed, or both, when comparing individuals or groups.

A Review of Mating Systems

The mating system of a population can be regarded as the ensemble of behaviors and physical adaptations specific for mating that are available to a population (Vehrencamp & Bradbury, 1984). The mating system of a population is an emergent property that reflects the traits and propensities of the individuals within the population (West-Eberhard, 1979). For example, characteristics of a monogamous mating

system among mammals include (1) the continual close proximity of an adult heterosexual pair during and outside periods of reproduction, (2) mating preferences, (3) the absence of unrelated adult conspecifics from the breeding pairs home range, and (4) breeding by only one adult pair in a family group (Kleiman, 1977). Other common forms of mating systems include polygyny, in which there is a prolonged association and essentially exclusive mating relationship between one male and two or more females at one time, and promiscuity, in which there is no prolonged association between the sexes and multiple matings by members of at least one sex (Wittenberger, 1979). The goal of research has been to identify the primary selective pressures or foci that have created the behavior differences associated with each mating system, with the use of appropriate theory and field work (Vehrencamp & Bradbury, 1984).

Puberty Modulation in House Mice (Mus musculus)

Most investigations of the phenomenon of puberty modulation have been conducted with house mice (Mus musculus). Researchers have identified several factors that can influence the timing of puberty in young female mice. Many studies have used indices such as the day of vaginal perforation or the detection of an estrous smear as a reliable means of assessing puberty. Considerably less research has been designed to investigate the factors that affect puberty in male mice. However, research with both

sexes appears necessary to understand how differences in the timing of puberty might affect the expression of social and mating systems.

A number of factors have been shown to influence the timing of puberty in female house mice. Broadly, these factors have included genetic differences (Drickamer, 1981), social conditions (see Drickamer, 1986 or Vandenbergh & Coppola, 1986 for recent reviews), and non-social factors such as temperature (Barnett & Coleman, 1959), photoperiod (Drickamer, 1975a), and season (Kruczek & Gruca, 1990). The current review and experiments have been based on the investigation of social factors that have been shown to influence puberty. Specifically, chemosignals or pheromones, found in the urine of several muroid species, have been shown to differentially accelerate or retard the attainment of puberty in young individuals (Levin & Johnston, 1986).

At least four types of urinary chemosignals can influence puberty in female mice (Drickamer, 1986). These signals include urine from (1) male mice, (2) pregnant or lactating females, (3) females in estrus, and (4) group-caged females. The first three signals accelerate the timing of puberty relative to female mice housed similarly but not exposed to the chemosignals. The last signal delays puberty in females compared to those not exposed to the signals. Below, I present the principal findings associated

with each of the four stimuli that affect puberty in female house mice.

Puberty Acceleration Caused by Male Urine

Not all urine from male mice is effective in accelerating the onset of puberty in females relative to those treated similarly but not exposed to the male urine. Dominant males release an acceleratory substance in their urine whereas subordinate males do not (Drickamer, 1983a). Similarly, prepubertal or castrated males do not release an acceleratory substance in their urine (Vandenbergh, 1969; Lombardi et al. 1976). Neither the specific type of housing condition nor the degree of genetic relatedness appears to mediate the effectiveness of the male pheromone directly. Drickamer (1983a) found that increasing the density of males does not alter the pattern of the chemical's release. In general, the effectiveness of the male chemosignal in causing pubertal acceleration in females is robust. Exposing females to amounts of male urine as small as 0.0001 cc per day effectively advances puberty in mice (Drickamer, 1982b, 1984a).

Despite the chemical's apparent acceleratory strength, such factors as the season or ratio of light-dark exposure can affect the female and thus indirectly affect whether the male's chemosignal advances puberty. The substance appears most effective in producing acceleration when it is collected from males during light onset and presented to females at this same time period (Drickamer, 1982a). The

effectiveness of the male chemosignal appears to vary because of changes in sensitivity of young female mice to the substance and not due to differences in the substance excreted by males (Drickamer, 1986). Both male and female mice have unique patterns of urine deposition that could influence the likelihood that puberty will be modulated in juveniles (Drickamer, 1989a).

Certain social conditions can influence the effectiveness of the acceleratory substance to induce puberty in females. Vandenbergh (1967) showed that the acceleratory effect is greater when males are in physical contact with females; contact-stimulation appears necessary for the effect to occur (Drickamer, 1974, 1975b; Bronson & Maruniak, 1975). The active chemical in male mouse urine appears to be relatively nonvolatile and under natural conditions it can influence females for some time after the male has deposited urine (Drickamer, 1986). The substance in male urine has been found to be effective in accelerating puberty within three days of exposure (Colby & Vandenbergh, 1974). Acceleration can be accomplished by exposing females to either male urine for two hours per day or to an intact adult male for one hour per day (Drickamer, 1983a). Thus, these findings show that although the age at which female house mice reach puberty can be reduced by the actual presence of an adult male, his presence is not necessary for the acceleration of puberty.

The male chemosignal appears to have its physiological influence in females via the vomeronasal-accessory olfactory system (Drickamer & Assmann, 1981). This system may be a common pathway affecting puberty modulation in various species. Ablation of the vomeronasal organ eliminates the ability of both mice and voles to receive pheromonal signals (Vandenbergh, 1988; Lepri & Wysocki, 1987). Other work investigating the physiological processes of male-induced puberty acceleration has been conducted but will not be reviewed here (see Reiter, 1982; Carter et al. 1986 for review).

Puberty Acceleration Associated with Pregnant and/or Lactating Females

Drickamer and Hoover (1979) first documented that the urine of pregnant or lactating females accelerates sexual development in female house mice. It is believed that the active chemosignal is the same that is produced by females in both reproductive conditions, because a number of experiments revealed no clear differences between the chemosignals (Drickamer 1986). Three days of exposure to urine collected from either pregnant or lactating females is necessary for puberty acceleration to occur in females that are less than 30 days old (Drickamer, 1984b). However, the substance does not cause acceleration in females when it is presented during the winter months. This lack of an acceleration in puberty appears due to differences in the sensitivity of young females to the cues that correspond to

changes in the season (Drickamer, 1986). The acceleration of puberty does not appear to differ as a function of kinship among females (Drickamer, 1984c).

Under certain conditions, urine from reproductively active females does not cause puberty acceleration in females. For instance, when reproductively active females are caged in groups or housed with non-reproductively active females, their urine becomes ineffective in causing either acceleration or delay in recipient females (Drickamer, 1983b). If young female mice are exposed to urine from both reproductively active females and urine from group-caged, non-reproductively active females, the exposed females are delayed in reaching puberty (Drickamer, 1982c; 1986).

The chemosignals associated with reproductively active females appear to be relatively volatile and are effective in causing acceleration when presented in volumes slightly larger than those of the male signal (0.03 cc of urine per day; Drickamer & Hoover, 1979; Drickamer 1982b, 1983c).

One difference between the acceleratory chemosignals of males and those of reproductively active females is that the chemosignals from females are effective in causing acceleration regardless of when they are collected or what time of the day they are presented to females. As noted previously, the urine from males is most effective in accelerating puberty when it is collected and presented to females during the onset of the light portion of the light-dark cycle (Drickamer, 1982a).

Puberty Acceleration Caused by Females in Estrus

Less is known about how the particular phase of the estrous cycle influences the chemosignals of the female donors that can influence puberty in other females. However, urine from singly-caged female mice in estrus decreases the latency until puberty is reached in young female mice. This effect is not seen when urine from single-caged diestrous females is presented to prepubertal females (Drickamer 1982c, 1984c).

The chemosignal in urine produced by estrous female mice is effective in advancing puberty in about 3 days and is effective when presented in small quantities such as 0.001 cc of urine per day (Drickamer, 1986). No differences in acceleratory effects were found when urine was collected from related or unrelated estrous females (Drickamer, 1984c).

Puberty Inhibition Caused by Group-Housed Females

Only one type of endogenously produced chemosignal has been found to inhibit or delay puberty reliably in female mice. Group-housed females produce a substance in their urine that delays puberty in other females. The time that estrous smears are detected in young female mice is delayed when they have been exposed to either soiled bedding of group-housed females or have had their nares painted with urine from group-housed females (Drickamer, 1982c).

Females exposed to the urine of group-housed females will begin to produce the inhibitory substance themselves.

The delay signal's effect occurs later than the effect of any acceleratory chemosignal. Specifically the delay signal requires four to seven days of exposure for inhibition to occur (Drickamer, 1977). Season also influences the degree of sensitivity young females have to this signal. Females are not delayed in reaching puberty when exposed to the substance during the summer, although during other seasons they are delayed in its presence (Drickamer, 1986).

Puberty Modulation in the Natural Environment

Although the phenomenon of puberty suppression or advancement is clearly established for house mice in the laboratory, it is appropriate to question whether similar processes occur within wild populations. A few studies have provided evidence that both puberty acceleration and suppression take place under natural conditions.

Massey and Vandenbergh (1980) conducted a series of experiments with populations of wild house mice enclosed within highway cloverleaf sections over a 2-year period. The urine from the females of two populations was collected via live-trapping during the spring, when population densities were low. This urine did not have an effect on the age of first vaginal estrus of laboratory-housed females. However, urine collected from the second population in December, when the population was crowded, significantly delayed the first estrus in females when compared with control females. Thus, the researchers

attributed the delay of puberty as being due to the increased density in the second population.

Urine collected from wild male mice of the highway "island" populations accelerated puberty in laboratory females by an average of seven days as measured by age at first estrus. Puberty acceleration occurred in response to the male urine despite changes in the season or population density at the time the urine was collected (Massey & Vandenbergh, 1981). Thus, it appears that male chemosignals and subsequent acceleration may be a more prevalent influence upon puberty in females than the delay chemosignal produced by female mice under crowded conditions.

In another set of experiments, acute population explosions were created by Vandenbergh and Coppola (1986) who introduced 40 second- or third-generation wild female house mice onto highway islands. This procedure allowed for a more critical test of the causal relationship between population density and the release of delay chemosignals. Urine samples were collected from females on each island during monthly intervals. Urine samples collected three weeks after the addition of the females onto the island populations caused an average delay in first vaginal estrus of 5.3 days in laboratory-reared females. Thus, this study provided additional evidence that the puberty-delay pheromone can be produced by females in response to acute increases in female density under natural circumstances (Vandenbergh & Coppola, 1986).

The Social Biology of Voles (Microtus)

A considerable amount of information exists on the social and mating systems of several species of voles (Microtus; see Wolff, 1985; Keller, 1985 for reviews). The social behavior of Microtus is both complex and variable (Wolff, 1985). Most species are active both during the day and night, and make use of above-ground runways as well as burrows. However, there are substantial differences in patterns of territoriality and of mating system. Below are listed some of the primary social differences among four species of Microtus, which are the four species studied in the following experiments. Broadly, their differences portray the degree of diversity within the genus Microtus.

Montane voles. Montane voles (M. montanus) are considered to commonly form a polygynous mating system, although facultative monogamy may occur at low densities (Jannett 1980; 1982). Both males and females typically defend exclusive territories against other same-sexed individuals (Jannett, 1982). The territories of males generally overlap the territories of one or more females. Thus, female montane voles appear typically to mate with only one familiar male, although no pair bond is formed (Wolff, 1985). Montane voles become more social and form aggregations during the winter months (Madison, 1984). The adult males of the species are typically larger than the females (males 35% larger; Dewsbury et al. 1980).

Meadow voles. The mating and social system of meadow voles (M. pennsylvanicus) differ in some respects from montane voles, and many data have been gathered on their social behavior (e.g., Madison, 1980, 1984; Webster & Brooks, 1981). Meadow voles appear to form a promiscuous mating system in which reproductively active females defend territories. In contrast to montane voles, the home ranges of males may overlap those of several other males as well as those of females (Wolff, 1985). Cases of one female mating with several males have been reported (e.g., Webster & Brooks, 1981). Meadow voles form aggregations during the late fall and winter months as do montane voles (Madison, 1984). The degree of sexual dimorphism in body weight is also relatively high, with adult males being larger than females (males 22% larger; Dewsbury, et al. 1980).

Prairie voles. In contrast to montane voles and meadow voles, prairie voles (M. ochrogaster) appear to be more social and form a monogamous mating system; a communal nesting group appears to be the basic year-round social unit (Getz et al. 1990). Evidence from the laboratory also suggests monogamy. Both the breeding male and female engage in extensive parental care of offspring (Getz & Carter, 1980), and male prairie voles failed to demonstrate the Coolidge effect when presented with a new estrous female after reaching sexual satiety (Gray & Dewsbury, 1973). Both males and females appear to be territorial and are aggressive to animals of the opposite sex. Both sexes of

breeding pairs defend a common home range that is approximately the same size (Gaulin & FitzGerald, 1988). The degree of sexual dimorphism in body weight is reduced in comparison to the dimorphism in montane voles and meadow voles, although the male is somewhat larger than the female (males 17% larger; Dewsbury et al. 1980).

Pine voles. Pine voles (M. pinetorum) also appear to be a highly social species and appear to live in communal groups (FitzGerald & Madison, 1983; Schadler, 1990). The mating system of pine voles appears to be monogamous, although cooperative polyandry has been suggested as an alternative system (FitzGerald & Madison, 1983). However, more recent evidence suggests that breeding is commonly restricted to the founding parents of a communal group (Schadler, 1990). The existence of a monogamous mating system had also been predicted to be most likely for pine voles among eight species of Microtus reviewed by Dewsbury (1981). Other evidence of monogamy includes male participation in the rearing of young, including the retrieval and brooding of infants (Shadler, 1990; Oliveras & Novak, 1986).

In contrast to many other species of voles, pine voles are almost entirely fossorial, and come to the surface only occasionally to feed (Wolff, 1985). Pine voles also appear to be territorial. FitzGerald and Madison (1983) found that each family had a discrete non-overlapping territory from other family groups. Differences in body weight between the

sexes appear minimal in pine voles (males 2% smaller; Dewsbury, 1990).

Puberty Modulation in Voles (Microtus)

Although several researchers have clearly demonstrated that puberty delay and acceleration occur in a few species of Microtus, such as prairie voles (M. ochrogaster; Carter et al. 1986) and California voles (M. californicus; Batzli et al. 1977), others have made weaker claims that such processes do or do not occur in other species of Microtus. For example, Jannett (1978) referred to field evidence that suppression occurs in montane voles (M. montanus) and Batzli et al. (1977) claimed that suppression does not normally occur in meadow voles (M. pennsylvanicus). Below, I review the primary literature regarding the four species of Microtus that were used in the later series of experiments.

Prairie voles. Most research concerning puberty modulation in Microtus has been conducted with prairie voles, where the presence of puberty suppression and acceleration are strongly supported (see Carter et al, 1986 for review). In an early study that indicated puberty delay occurs in prairie voles, Hasler & Nalbandov (1974) examined pairs of weanling females caged with males with different characteristics. Weanling females that were caged with a littermate male had significantly longer latencies until vaginal opening and production of a first litter than compared to females that were caged with either a non-littermate male or an adult male. The range of days

until vaginal opening occurred among these groups was substantial. Females kept with littermate males became perforate 30 days after pairing, on average, compared to females paired with nonlittermate males that became perforate eight days after pairing.

Batzli et al. (1977) found evidence that both sexes of prairie voles could be suppressed developmentally, as assessed by differences in body weight, when they were housed with littermates. During this period, females remained vaginally imperforate and males had abdominal testes. After siblings were paired with unfamiliar adults of the opposite sex, rapid increases in body weight and subsequent reproduction occurred.

The stimuli necessary for the acceleration of puberty in prairie voles have been studied. Young female prairie voles, when exposed to a sexually experienced male for a 1 h period, showed significant increases in uterine weight as soon as 2 days after exposure (Carter et al. 1980). Drops of male urine applied to the upper lip of young females caused significant increases in uterine weight compared with females caged alone, with a female sibling, a castrated male, or exposed to the urine of a castrated male.

In another series of experiments, kinship per se was shown not to be a limiting factor for the advancement of puberty in prairie voles (Carter et al. 1980). When urine from male siblings was applied to the nares of females for 6 consecutive days, it led to significantly heavier uterine

weight in females, compared to females housed with either a sibling male or with a sibling male and treated with water placed on the nares.

Together, the results of studies with prairie voles suggest that a chemical agent or pheromone present in male urine can be passed from male to female by direct contact. From behavioral observations, Carter et al. (1980) suggested that the active male pheromone was transmitted by naso-genital contact between the sexes. However, sibling prairie voles rarely engage in naso-genital investigation and thus the lack of investigation between siblings may function as a barrier to reproductive activation and incestuous matings (Carter et al. 1986).

Although it is not clear how siblings inhibit reproduction in other siblings, young female prairie voles appear to be a primary source of reproductive suppression for other male and female siblings. Getz et al. (1983) found that for 15 suppressed litters, all but 1 of 32 females (or 97%) reared with sibling females were suppressed, while only 3 of 15 (20%) reared with sibling males were suppressed. A similar finding was apparent in males; 10 of 11 males (91%) reared with sibling females were suppressed and only 3 of 9 males (33%) raised with sibling males were suppressed. The results suggest that for prairie voles, the sex of siblings can have a substantial effect upon the timing of puberty.

Additional work with prairie voles has revealed that the suppressive effect of female presence appears largely due to chemosignals present in their urine (Getz et al. 1983). In this study, virgin prairie voles were first reproductively stimulated by exposing them to an unfamiliar sexually experienced male for a one-hour period, then the females were housed in a variety of conditions. The uteri were weighed 48 hours later. The uterine weights of females that had urine from either a female sibling, a virgin non-sibling, or a pregnant female, placed on their nares did not differ significantly from the uterine weights of non-stimulated females. The only significant increase in uterine weight was in the group of females that were stimulated by the male and then maintained alone. Thus, the results indicate that urine from female prairie voles is effective in counteracting or suppressing puberty in female prairie voles.

Although it is not known how the chemosignal is transmitted between females, activation by the non-volatile male acceleratory pheromone appears to be caused by mutual anogenital investigation between the sexes (Carter et al. 1980). Hofmann and Getz (1988) found that virgin prairie voles that were exposed frequently to unfamiliar males could "override" the reproductive suppression typically experienced by females that remained within a family group. Thus, it seems plausible that under natural conditions where density is dramatically increased, such as during the growth

phase of a population cycle, normal suppression can be counteracted by male presence. In support of this argument, Getz and Hofmann (1986) found that of free-ranging prairie vole females that remained at the natal nest at low population density, 18% were reproductively active, while 77% became reproductively active at high density. All females that dispersed from the natal nest were found to became reproductively active.

Pine voles. Both puberty delay and acceleration appear to occur in pine voles, M. pinetorum (Schadler, 1983; Lepri & Vandenbergh, 1986). Schadler (1983) presented evidence that male siblings are an important source of reproductive inhibition for female siblings. Mating was inhibited between a female and an unrelated male, while a male sibling was sequestered behind a wire mesh barrier. Other evidence of puberty delay was shown by Lepri and Vandenbergh (1986) when they placed young female pine voles with adult males into two type of cages: one with clean bedding or another with bedding that had been soiled for two weeks by the female's family. Forty-eight hours later, the females' uteri and ovaries were removed and weighed. The uteri and ovaries of those placed in the clean cage were significantly heavier than those placed in the cage soiled by the family.

In another experiment, Lepri and Vandenbergh (1986) demonstrated puberty acceleration in pine voles by exposing 4-week-old females to feces and urine from animals in one of the following groups: (1) intact males, (2) castrated males,

(3) singly-housed females, (4) group-housed females, or (5) control (clean cage). When uterine and ovarian weights were compared, results indicated significant increases in both organs from females exposed to the stimuli from the males of either groups compared those from the control females. No significant differences were found when the organs of the control group were compared to those in the two groups that received female stimuli.

Meadow voles. Although there is evidence that pubertal acceleration occurs in female meadow voles (Baddaloo & Clulow, 1981), the evidence is equivocal for any type of puberty delay. Pasley & McKinney (1973) presented evidence that females caged in groups of eight had smaller ovaries and uteri than singly-housed females. However, it is possible that this type of sexual suppression was due to overcrowding and stress and not due to pheromones. Batzli et al. (1977) reported they did not find suppression to occur in five of six litter-housed groups of meadow voles. Additional data are needed to determine if sexual suppression occurs in meadow voles as a result of pheromones and not due to overcrowding and stress.

Montane voles. Fewer reports of pubertal acceleration or delay can be found scattered in laboratory and field reports for other species of Microtus. Field evidence suggests that pubertal delay occurs in montane voles (Jannett, 1978), although a critical test of this phenomenon has not been reported. Recent laboratory evidence has shown

that puberty acceleration occurs in female montane voles when exposed to males. Sawrey and Dewsbury (1991) found shorter latencies until vaginal perforation and first cornified (estrous) smears in females housed across a wire-mesh barrier from males, as compared with females without a male present. Controlled studies are needed to determine if puberty delay occurs in montane voles when they are exposed to pheromones.

It is possible that not all species of Microtus show both delay and acceleration of puberty, or at least are not affected to the same degree by comparable stimuli. Evidence suggests that suppression does not occur, or occurs only weakly, in female tundra voles (M. oeconomus) when they are housed with male siblings (Facemire & Batzli, 1983). Batzli et al. (1977) reported morphological differences between California voles and prairie voles. California voles that were housed with littermates grew more slowly than controls, although females became perforate and males became scrotal at about the same time in the littermate group as did the controls (30 days). This pattern differed from prairie voles in which males typically developed abdominal testes and females remained vaginally imperforate when littermates were held together. In both species, however, reproduction by littermates was usually delayed until they were paired with unfamiliar animals. Thus, there may be a variety of physiological and behavioral differences related to puberty modulation among various species of Microtus. The

illumination of the similarities and differences in patterns of puberty modulation may prove instrumental in understanding the dynamics of their social and mating systems.

Puberty Modulation in Male Voles (Microtus)

Considerably less research has been conducted on the various factors that can influence puberty modulation in male Microtus, although cues from males may be critical for influencing puberty in other siblings among some species. Evidence for California voles suggests that male siblings influence the sexual development of one another. Batzli et al. (1977) found that the growth rates of males were suppressed when they were paired with another male sibling, although growth rates were not affected when they were paired with a female, a male non-sibling, or with a female sibling.

Other evidence suggests that the odors from family members of California voles suppress male pubertal development. In this species, the odors from mature males or sires did not appear either to accelerate or inhibit the sexual development of males (Rissman et al. 1984). However, male California voles that were reared in the presence of family bedding material and subsequently paired with another non-relative female failed to cause increases in uterine weight within four days (Rissman & Johnston, 1985). They suggested that the lack of stimulation in the females was due to low levels of circulating androgen in the males.

Thus, odors associated with a reproductively active female might be one source of pubertal suppression in male California voles.

A few anecdotal accounts of puberty modulation among males of other species of Microtus exist. For example, juvenile male common voles (M. arvalis) have been reported to show reduced testicular development when housed near cages with crowded adults (Lecyk, 1967). In summary, it appears that although males of various species of Microtus may be reproductively modulated by specific stimuli, the limited data make conclusions premature. There is a paucity of information regarding pubertal modulation in male voles, and for males of other rodent species as well. It seems worthwhile to correct this bias with future studies.

Principles Underlying Puberty Modulation in House Mice and Voles

There has been little effort to integrate the large body of literature into an effective theoretical framework to study the relationships between primer pheromones, age at first reproduction, and demographics (Vandenbergh & Coppola, 1986). Many of the existing hypotheses regarding puberty modulation are based upon studies of house mice (Mus musculus) because most of the research has been conducted with this species. Vandenbergh and Coppola (1986) suggested that some inferences may be applied to other species, such as voles. However, they caution that given the differences in the reproductive biology among genera, it seems unlikely

that the selective pressures influencing puberty modulation will be identical across them. Observed similarities among genera or species must be interpreted carefully when attempting to formulate general principles.

Below I list some of the most prominent frameworks that appear useful for integrating our understanding of the causes and functions of the pheromonal effects upon puberty modulation found among species such as house mice and voles.

A Life-History Theory of Puberty Modulating Pheromones

One of the broadest theoretical frameworks to investigate and interpret patterns of puberty modulation is the life history theory proposed by Vandenbergh & Coppola (1986). According to this approach, life history tactics are evolved sets of coadapted traits designed to solve particular ecological problems (Stearns, 1976). Whether animals typically reach sexual maturity relatively early or late, all are viewed to have been selected to maximize lifetime reproductive success.

Selective factors favoring puberty acceleration. Life-history theory predicts that for increasing populations, or those with large fluctuations or repeated episodes of colonization, early maturity will be favored (Vandenbergh & Coppola, 1986). Such characteristics describe feral house mouse populations. In general, it is believed that selection will favor early and total investment by individuals to produce the maximum number of young whenever the environment is highly favorable for

reproduction. They note that previous theories (e.g., Cole, 1954; Lewontin, 1965) have indicated that in a rapidly expanding population, the age at first reproduction should be driven to the physiological minimum by natural selection. Species that possess a combination of life-history traits including an early age of first reproduction, the production of many young, and semelparity have been classified as being "r-selected" (MacArthur & Wilson, 1967; Pianka, 1970). This combination of traits is often present in species that experience rapid population growth in favorable environments.

Several factors are thought to favor early reproduction (Vandenbergh & Coppola, 1986). First, early reproduction will be favored as reproductive costs decrease (Schaffer & Elson, 1975). Reproductive cost is the deleterious effect of present reproduction on future survival and/or fecundity. Early reproduction will also be favored when the reproductive value of individuals decrease as they grow older (Gadgil & Bossert, 1970). These patterns suggest that early reproduction will be favored when it does not negatively influence a female's ability to reproduce later in life and thus lower lifetime reproductive success.

Delayed reproduction (puberty) in house mice. The function of delayed reproduction or puberty is more obscure than the function of puberty acceleration (Vandenbergh & Coppola, 1986). They suggest that it is difficult to explain why opportunistic species, such as house mice,

benefit from delaying puberty and reproduction. However, whatever the function(s) of puberty delay, it has been found in several mammalian species (Vandenbergh & Coppola, 1986).

There appear to be general conditions that favor delayed reproduction. Delayed reproduction could evolve if individuals gained fecundity or produced better quality offspring (Vandenbergh & Coppola, 1986). In general, it is believed that many of the demographic or environmental factors that favor delayed reproduction are the opposite of those favoring early reproduction. Some have suggested that delayed reproduction would be selected in stable populations at or near the carrying capacity of the environment (Cole, 1954; Lewontin, 1965) or in declining populations (Hamilton, 1966; Mertz, 1971). Selection in saturated environments, which favors the ability to compete and avoid predators, has been referred to as "K-selection" (MacArthur & Wilson, 1967; Pianka, 1970). Traits correlated with this type of selection often include late maturity, the production of few and large offspring, long life spans, and extended parental care.

There are a few causal factors that are thought to underlie delayed reproduction (Vandenbergh & Coppola, 1986). For example, as the reproductive costs increase, in terms of adult mortality, and as the reproductive value an individual can accrue by not reproducing increases with age, delayed reproduction will be favored. If reproductive success is

contingent upon age, size, or social status, delayed reproduction is also favored (Geist, 1971).

Despite the general conditions that have been proposed to predict under what circumstances early or late puberty will occur, the current state of life-history theory allows us to draw only rather vague and imprecise conclusions about the timing of reproductive maturity (Vandenbergh & Coppola, 1986). Despite this drawback, life-history theory provides a broad theoretical background from which to interpret new information. In addition to the previous conditions which favor puberty acceleration or delay, there are a few additional principles that may guide our understanding of these processes. Below, I summarize two of the most prominent themes which have emerged.

Puberty Modulation (Mutualism (Cooperation) versus Conflict)

One assumption that underlies most explanations for the evolution of puberty modulation is that puberty modulation is either of mutual benefit to the sender and receiver of pheromones or that the sender and receiver of the pheromones are in some form of conflict.

Examples of puberty modulation in the context of cooperation. Bronson (1979) proposed that adult male mice and adult or prepubertal females both gain in fitness through mutual stimulation of reproductive acceleration via pheromonal transfer. In this hypothesis, male urinary pheromones cause the release of LH in recipient females that speeds the attainment of puberty and ovulation.

Reciprocally, female pheromones cause increases in LH in males which ultimately leads to increases in testosterone and additional pheromone synthesis in males. Such a system could greatly enhance the speed at which males and females could reproduce, thus benefiting both sexes of breeding pairs.

A second proposed benefit of mutualism was raised by Vandenbergh and Coppola (1986) who proposed that the detection of the puberty-delay and acceleratory pheromones produced by adult female house mice provides information to young females about the general quality of the environment for reproduction. Presumably under crowded conditions, adult females release puberty delay pheromones that benefit both the sender and receiver to postpone reproduction. In more favorable environments for reproduction, the release of pheromones that accelerate puberty by females would hasten the speed at which puberty and reproduction would occur among female offspring. Presumably, the young females, their mates, and kin would benefit from more optimally timed reproduction.

A third example where mutual benefits may occur between the pheromone senders and receivers is between parents and offspring in some cooperatively breeding species (Emlen, 1984). Cooperative breeding refers to any situation when more than two individuals provide care in the rearing of young (Emlen, 1984). In this example, puberty delay and

inbreeding avoidance may have been co-evolved traits in species where cooperative breeding occurs.

It appears that certain ecological constraints, such as a saturated habitat, favor the retention of subadult individuals within a family group, at least until conditions enable independent reproduction. Emlen (1984) suggested it is possible that when the probability of successful dispersal and independent breeding are low, average fitness may be increased by remaining in a group until group size reaches some optimum. Other benefits that may occur in cooperatively breeding groups include: (1) benefits to helpers that gain breeding experience; (2) inheritance of the parental territory; (3) dispersion in groups when competition for reproductive vacancies is strong; and (4) some form of reciprocity may take place, such as when one individual forgoes breeding and helps another rear offspring with likelihood of return aid at a later point in time; and (5) increased inclusive fitness through aiding in the rearing of close relatives (Emlen, 1984). Thus, both the direct and indirect components of inclusive fitness might be increased via reproductive suppression when accompanied by helping behavior.

A final proposal for mutualistic benefit of reproductive suppression, or the lack thereof, in voles has been proposed by Christian (1970). Christian (1970) emphasized the selective pressures exerted by the habitat where a particular species evolved. Species such as meadow

voles, which evolved in habitats that were patchy and ephemeral, such as moist meadows, evolved mechanisms that aided in the colonization of newly created habitats. Thus, mechanisms that led to successful dispersal were adaptive for all members. He proposed that a density-dependent endocrine response that led to increased aggression with subsequent dispersal would serve this function. Hence, a lack of sexual suppression among siblings would be expected in species that evolved under these circumstances.

In contrast, species such as prairie voles that evolved in the continuous and extensive habitats of the great plains of central North America were believed to have evolved mechanisms that enabled a greater level of social tolerance and be more sensitive to reproductive inhibition (Christian, 1970). It is plausible that mechanisms to inhibit incestuous mating among family members might be most evident in highly social species.

Puberty modulation in situations of conflict. Different researchers have proposed instances where there appear to be conflicts of interest between pheromone senders and receivers. Bronson (1979) proposed there was an antagonism between female house mice to inhibit puberty and reproduction. Such antagonism between females may be accomplished either directly through aggression or through the production of delay pheromones.

Wasser and Barash (1983) proposed a similar hypothesis. They stressed the role of female-female competition to

reproduce as a key driving force to explain the high rates of general reproductive failure among the females of many species of mammals. The antagonistic pheromonal influence of reproduction between female prairie voles conforms to their theory (Getz et al. 1983)

Competition among the males of some species via pheromones may also be present in some species. Although Bronson (1979) noted the high rate of aggression observed between territorial male house mice and other males, he did not discuss pheromonal interactions among them. Vandenbergh (1971) found that adult male house mice had an inhibitory effect on the reproductive development in young males, while the presence of adult females accelerated their development. The possibility that males may inhibit puberty and reproduction of other males in other species through pheromonal communication should remain open for continued investigation.

Socially-Dependent Versus Socially-Independent Systems

Another framework within which to view and investigate the reproductive influences of pheromones on the timing of puberty can be called the socially-dependent versus socially-independent dichotomy. The essence of this framework is that some species of Microtus appear to be more dependent upon the direct exposure of other animals in order to become reproductively mature and active. Taylor (1990/1991) characterized two types of estrus induction patterns among females of different species of Microtus. He

provided evidence that the highly social prairie voles, could be characterized as being male-dependent. Females must typically be exposed to stimuli from males for a relatively long period of time before estrus is attained. In contrast, other less social species, such as meadow voles and montane voles, were referred to as being male-independent (Taylor 1990/1991). Species of this type do not attain estrus totally independent of male stimulation, but rather are much less dependent on direct male contact than are male-dependent species.

Puberty Modulation as an Artifact

An alternative viewpoint of the phenomena of puberty modulation is that the effects are some form of laboratory artifact. Examples of puberty modulation must be evaluated for the possibility that differences in the timing of puberty could be the result of close confinement with other animals, unnaturally high densities, or a product of artificial selection (see Bronson, 1979; Vandenbergh and Coppola, 1986).

Bronson (1979) suggested that while the mechanisms underlying the phenomena in question may be real, it is possible that the mechanisms evolved to serve one adaptive purpose in the wild, yet find expression in other ways in the laboratory environment. Vandenbergh and Coppola (1986) have argued against the proposal that puberty modulation is some form of laboratory artifact in house mice. First, they suggested that the delay of puberty in female mice which

results from a specific stimulus such as a urinary cue suggests an evolved signalling function that must have some adaptive value. A second reason is that the general social context in which puberty delay occurs in the laboratory is known to occur in the field. Descriptions of the social organization of commensal mice generally fit the conditions necessary for pubertal suppression to occur. The last line of evidence comes from the field studies conducted with wild house mice confined to clover-leaf highway populations (Massey & Vandenbergh 1980; 1981, see prior section). These studies show that at least under some conditions, the production of delay and acceleratory pheromones can occur in animals from free-ranging environments. Nevertheless, additional studies and further demonstrations that puberty modulation occurs among mice and other species such as voles in the field or under semi-natural conditions seem warranted.

Summary of Principles of Puberty Modulation in House Mice and Voles

From the primary findings and related principles presented for puberty modulation in species such as house mice and voles, it is clear there is no all-encompassing theory that leads to clear predictions when puberty should and should not occur, or to the specific stimuli that should cause modulation, and the specific function(s) it serves. However, the emerging picture is that a variety of stimuli are capable of influencing puberty and multiple functions

might be served by puberty modulation among various species. Vandenbergh and Coppola (1986) suggested that discovering the role of priming pheromones in the interactive process of life-history and behavioral adaptations will require the "melding" of empirical and theoretical points of view.

Problems with Previous Studies of Puberty Modulation with Voles

Scattered reports indicate that puberty modulation occurs in a variety of species of Microtus. Some species have been studied in considerable detail (e.g., prairie voles, see Carter et al. 1986). However, several sources of confusion have hampered our ability to explore possible relationships between puberty modulation and the expressed differences in the social and mating systems among Microtus. First, the lack of common procedures and measures used by various researchers appear to be significant, although reasonably easy, problems to correct. A variety of measures and procedures have been used to assess the timing of puberty, ranging from the measurement of body weight to analyses of hormone binding sites within the brain. Such diversity of procedures make meaningful comparisons among species difficult. For example, Dewsbury (1981) attempted to evaluate the usefulness of several proposed correlates of monogamy to predict its presence in several muroid rodents. However, he deemed it was not possible to compare meaningfully the existing reports for patterns of sexual maturation across several species. Thus, with such

variation evident for sexual maturation among muroid rodents, future studies with identical procedures appear necessary to make useful comparisons among species.

A second problem is the lack of proper controls that are necessary to identify the effective stimuli influencing puberty among species. Cues such as the presence or absence of an adult male or sire, the presence and number of opposite-sexed siblings, and the presence or absence of a reproductively active female have all been found to be effective stimuli that can influence the timing of puberty in one or more muroid species. Unfortunately, in many studies such cues are not systematically controlled or reported. The use of soiled bedding that is transferred from one cage to another provides one means of critically testing the assumption that pheromones produced by one or more individuals cause puberty modulation in others. Such a procedure controls for the potential influence that nonolfactory stimuli, such as visual or auditory, could have upon puberty modulation.

Finally, the database, which is a prerequisite to determine the general patterns or principles of puberty modulation, is still relatively small and, in some cases, misrepresentative of the genus Microtus. For example, although only a few studies have provided evidence of puberty modulation in some species of Microtus such as montane voles (e.g., Sawrey & Dewsbury, 1991), substantial data exist for other species, such as prairie voles (Carter

et al 1986; Getz et al 1983). In addition, most studies on Microtus, like those for mice (Mus), are female-biased. Most studies have been designed to determine how olfactory and social cues affect puberty in females rather than males of various species. Ideally, the factors influencing the onset of puberty would be studied concurrently for both sexes. Such patterns should be viewed for the possibility of significant interactions occurring between the sexes of a given species.

Thus, whereas the present list of problems encountered with studies of puberty modulation in Microtus is not exhaustive, it outlines significant sources of ambiguity and deficiencies in prior studies. Fortunately, with standard procedures and proper controls used with a number of species, a greater understanding of how puberty modulation can impact the formation of contrasting social and mating systems among Microtus might be possible.

CHAPTER 2
GENERAL METHODS: SUBJECTS, HOUSING, AND APPARATUS

Four species of voles (Microtus) were studied in each of three experiments. Species studied included pine voles (Microtus pinetorum), prairie voles (M. ochrogaster), meadow voles (M. pennsylvanicus), and montane voles (M. montanus). Breeding colonies of each species were maintained in the Psychology Building at the University of Florida. These facilities were accredited by the American Association for Accreditation of Laboratory Animal Care (A.A.A.L.A.C.).

All subjects were born to breeding pairs that were laboratory-reared and that had been derived from wild populations within the United States. Efforts were made to maintain genetic diversity while guarding against possible inbreeding in all species. All species were kept in separate colonies that were housed in windowless and air-conditioned rooms that were maintained on a reversed 16:8 light-dark photoperiod with light onset at 2000 hr. All colonies contained animals of both sexes throughout the experiments on a combination diet of Rabbit Chow (Purina Mills) and Laboratory Rodent chow #5001 (Purina Mills) with water available ad libitum. In an effort to promote continuous breeding and a sufficient supply of subjects, a handful of lettuce was given to breeding pairs on a weekly basis. Breeding animals used in Experiment 3 similarly

received these weekly supplements, although no subjects used in Experiments 1 and 2 received lettuce supplements during the duration of their test phase.

All subjects were maintained in large 48 X 27 X 13 cm polycarbonate cages unless they were housed individually, in which case they were housed in 29 X 19 X 13 cm polycarbonate cages. All subjects used in the studies were used in only one experiment.

CHAPTER 3
EFFECT OF OLFACTORY CUES UPON PUBERTY IN FOUR SPECIES OF VOLES (EXPERIMENT 1)

Rationale

Experiment 1 was designed to determine if puberty delay and acceleration occur solely as a function of the presence of olfactory cues (i.e., pheromones) that are contained in the soiled bedding in each of four species of voles Microtus. In several studies where puberty modulation has been reported, researchers have either housed animals directly with or across from other animals (e.g., caging juvenile females with, or across a wire-mesh screen from, adult males). Although olfactory cues and pheromones are believed to be key stimuli that produce changes in the timing of puberty, many procedures have not been designed to exclude the possibility that other stimuli critically affect differences in the timing of puberty (e.g., visual, auditory, or somatosensory cues).

The use of soiled bedding, which is transferred between donor and recipient animals, has been shown to be one effective method to expose animals to pheromones while controlling for exposure to other non-olfactory stimuli that could influence sexual maturation. Soiled bedding has been used successfully to reveal changes in reproductive development among house mice (Drickamer, 1982c), pine voles

(Lepri & Vandenbergh, 1986), and California voles (Rissmann et al. 1984; Rissmann & Johnston, 1985).

Measures and Predictions

Specific changes in the physiology of male voles that would indicate sexual suppression would include reproductive organs that weighed less than the organs of subjects exposed to the clean bedding. In addition, the anogenital distance among males considered suppressed would be smaller than the anogenital distance in those exposed to clean bedding. Measures that would reflect sexual suppression in female voles, would include delayed latency until vaginal perforation and smaller percentages of cornified cells in the vaginal smears compared to those exposed to clean bedding. Among several species of voles, higher proportions of cornified cells are associated with a higher incidence of estrus and sexual receptivity (Sawrey & Dewsbury, 1985; Taylor et al. in press). In contrast to indices of sexual suppression, indices of sexual advancement or acceleration would include the opposite changes to those listed above for sexual suppression.

Because of prior evidence that the highly social female pine voles and prairie voles typically require direct male contact for full reproductive activation (e.g., Carter et al. 1987; Schadler & Butterstein, 1979), I predicted these species would not be affected, or only minimally, when exposed to the soiled bedding material. These species may be considered socially dependent for reproductive activation

(i.e., socially-dependent or "male-dependent" species, see Taylor, 1990/1991).

In contrast, the females of the less social species (i.e., socially-independent species), meadow voles and montane voles, have been shown to become sexually receptive with little or no previous direct exposure to males or to show marked changes in reproductive physiology through exposure to male urine alone (Taylor, 1990/1991; Baddaloo & Clulow, 1981). Field evidence has suggested that both male and female montane voles are sexually suppressed when they remain in natal family groups under high densities (Jannett, 1981). Thus, meadow voles and montane voles were predicted to show marked responses to the presence of the soiled bedding material. Specifically, they were predicted to be sexually suppressed when exposed to odors from the natal family or from adults of the same sex, whereas they would be sexually accelerated when exposed to odors from unfamiliar opposite-sexed individuals. Young animals not exposed to odors from others (e.g., clean bedding) were expected to show intermediate rates of growth relative to the rates of those in the other conditions.

Method

Subjects

A total of 514 animals were used in Experiment 1, 256 were male and 258 were female. All were born to existing laboratory stock maintained at the University of Florida. The four species included pine voles (*Microtus pinetorum*),

prairie voles (M. ochrogaster), meadow voles (M. pennsylvanicus), and montane voles (M. montanus). All measures were recorded between September 1990 through November 1991.

Procedure

Animals from each of the four species of Microtus were weighed and individually caged in 23 X 19 X 13 cm polycarbonate cages at three weeks of age (day 21-22). Individuals were assigned randomly (via a random number table) to one of four conditions: (1) "Family" subjects received transfers of soiled bedding from their family group (groups containing an adult male, female, and subsequent offspring) every other day; (2) "Male" subjects received soiled bedding from a pooled sample that was derived from the cages of five unfamiliar adult males (see additional details below); (3) "Female" subjects received soiled bedding from a pooled sample of five unfamiliar adult females; and (4) "Control" subjects received transfers of clean wood-chip bedding that had been placed in a vole-free colony room and exposed to air as the bedding within the cages of animals had been exposed.

Subsequent offspring born to the breeding females that supplied the bedding in the Family condition were not reduced in number and were removed from the group at three weeks of age (21-22 days old). Additional litters born into the family groups were treated in the same manner. No more

than two animals of the same sex and derived from the same litter were used in the same condition.

All subjects were kept in homospecific colony rooms and maintained as outlined in the general procedures. Animals in each of the experimental conditions were housed in small cages and maintained on separate shelves in each of the colony rooms in order to minimize the exchange of olfactory cues among subjects in the various conditions.

Bedding transfers and maintenance. Bedding transfers occurred on the first day of placement into the experiment (week 3) and then on every other day throughout the 6-week test period. Bedding samples were collected and distributed into recipient cages during the first four hours of light onset (2000-2400 h). Pooled bedding came from the cages of five unfamiliar, individually-housed adult animals of the appropriate sex that did not have their bedding removed for four days. Bedding samples were mixed and transferred into the cages of subjects in 200 cc volumes with the use of plastic cups that were washed with mild detergent after each use. All donor cages were cleaned weekly. Cleaning involved the replacement of all soiled bedding with clean bedding, except for the retention of 800 cc of soiled bedding within each family cage and 400 cc in each individually-housed animal to maintain some common olfactory cues available to the residents. Subjects that received transferred bedding had 200 cc of bedding removed from their cages on the day of exchange and had 200 cc of the

appropriate type of bedding added to replace the lost volume.

Pooled bedding was used in the "Male" and "Female" conditions to provide a more uniform stimulus to animals in these conditions. It seemed plausible that some of the bedding-donors would produce soiled bedding that differed in stimulus quality from others. For example, females may have excreted different amounts of metabolites in their urine as a result of fluctuating hormone levels. Previous researchers have used similar pooling methods in behavioral preference tests with house mice (e.g., Coppola & O'Connell, 1988; Drickamer 1989b).

Physiological and morphological measures. Body weights were recorded for subjects when they were weaned (day 21-22) and at weekly intervals until they were 56 days of age (9 weeks). By this time, individuals from each species would be considered to have reached adult status under standard laboratory conditions, with the possible exception of pine voles. Lepri and Vandenbergh (1986) found that the median age for male pine voles to sire a litter was 57 days (North Carolina population), whereas the median age for first conception among the females was 50 days.

Other measures of puberty included the day of vaginal opening for females and the anogenital distance of males at weekly intervals. Vaginal smears were taken daily from each female beginning on the day they first became perforate. All other subjects, including males, were handled daily in a

similar fashion as were females, as a control procedure for the effects of handling. During all handling procedures, subjects were held with individually-assigned vinyl gloves in order to prevent the transference of odors among subjects.

At the end of the 9-week period, subjects were euthanized and selected organs were removed and weighed to the nearest 0.1 mg. Organs weighed included the uterus, ovaries, and adrenal glands of females and the seminal vesicles, testes, and adrenal glands of males. All dissections and weighing of organs were conducted by the author who used uniform procedures. In all cases, efforts were made by the author to remain blind to the condition of the subjects prior to the dissection procedure. This was accomplished by dissecting animals in small groups if possible. During this procedure, the identification cards of subjects were placed upside-down and beneath the individual trays that held the removed organs from each subject. The organs were covered with moist paper towels in a uniform fashion. The author would then scramble the position of the dissection trays just prior to the weighing procedure. Thus, in most cases, the author was blind to the experimental condition of the animals during the final cleaning and weighing procedure.

Statistical Analysis

Analysis of variance procedures (ANOVA's) were used to assess the effects of treatment on subjects. All data were

transcribed to computer spreadsheets and analyzed with the CSS: Statistica software program (StatSoft, Inc). Data were analyzed independently for each species and sex, because of occasional instances of heterogeneity of variances between the species. Analyses consisted of either one- or two-way ANOVA's with the experimental condition comprising the primary between-subjects factor. Some measures, such as body weight, were recorded over the course of the study and were analyzed with the additional repeated-measure factor of weeks. Paired organ weights were analyzed with the repeated-measure factor of body location (left versus right), because of known physical asymmetries among some paired organs (e.g., Pinter, 1968) or other possible functional asymmetries (e.g., Clark & Galef, 1990). The alpha level was held at .05 in all comparisons, and all comparisons were based on a two-tailed probability.

Results

Results of each of the measures are reported below for the effect of the experimental conditions separately for each species. The statistical values of ANOVA's are reported directly in the text or in specified tables, although the exact probability values of post-hoc comparisons (Neuman-Keuls tests) are not reported in order to streamline the text. Only post-hoc comparisons that were significantly different (p's $< .05$) are discussed in the text, or are clearly specified as not being significant when discussed.

Body Weight

Generally, the experimental condition (bedding type) did not substantially influence body weight among the species. However, male pine voles were significantly affected by the condition (see below).

Because only the factor of week was statistically significant among the other species, simply reflecting increases in body weight across age, analyses for each species are located in Table 3-1 (see Appendix A for means at each week).

Pine voles: males. Post-hoc analyses revealed that male pine voles in the Family condition (22.71 $\pm$ .53, N = 16) and Male condition (22.96 $\pm$ 1.05, N = 14) weighed significantly more than those reared in either the Control condition (21.24 $\pm$ .53, N = 17) or Female condition (20.64 $\pm$ .65, N = 16) at week 9 (interaction of condition and week, $F(18, 348) = 1.98$, $p = .010$)(see Figure 3-1). The main effect of week was significant, $F(6, 348) = 600.87$, $p = < .001$), indicating larger body weights across weeks, although the main effect of condition was not significant, $F(3, 58) = 1.68$, $p = .181$ (see Appendix A for means at each week).

Anogenital Distance

The anogenital distances of the male prairie voles, meadow voles, and pine voles were not substantially influenced by the condition. However, there was some

indication that anogenital distances among male montane voles were influenced by the condition (see below).

Because only the factor of week was statistically significant in the other species, simply reflecting increases in anogenital distance with increasing age, analyses for all other species are located in Table 3-2 (see Appendix B for means at each week).

Montane voles. Although a significant interaction of anogenital distance by week of measurement was found, $F(18, 360) = 1.84$, $p = .019$, post-hoc comparisons did not reveal significant differences among any of the groups (see Table 3-2 for means at week 9 and ANOVA results; complete means for each species and week are located in Appendix B). The mean anogenital distance of montane voles in the Control condition ($M = 13.44 \pm .44$ mm, $N = 16$) approached being significantly larger than compared to that in the Family condition ($M = 12.25 \pm .31$ mm, $N = 16$ ($p = .09$). The mean anogenital distances in the other conditions were intermediate in value to those in the Control and Family conditions. The main effect of week was significant, $F(6, 360) = 173.00$, $p < .001$), although the main effect of condition was not, $F(3, 60) = 0.33$, $p = .798$).

Adrenal Weight

Adrenal weights were minimally affected by the condition among all species and sexes, with the exclusion of male pine voles (see below). The mean adrenal weights and

analyses for all species, when uncorrected and corrected for differences in body weight, are located in Table 3-3.

Pine voles: males. The condition did not significantly influence the adrenal weights of male pine voles, when uncorrected for differences in body weight, $F(3, 59) = 1.26$, $p = .296$ (see Table 3-3 for means and ANOVA results). However, the analysis of adrenal weights, corrected for differences in body weight, revealed significant differences among the conditions (see Figure 3-2). Post-hoc comparisons revealed that the adrenals of males in the Female condition ($M = 24.78 \pm 1.17$, $N = 16$) were significantly heavier than in the Control condition ($M = 21.60 \pm 1.11$, $N = 17$) or Family condition ($M = 20.06 \pm 1.35$; $N = 16$)(main effect of condition, $F(3, 59) = 3.32$, $p = .026$). The adrenals from males in the Male condition were similar in weight to those from the Control and Family conditions but failed to differ significantly from those in the Female condition ($M = 21.02 \pm 1.09$, $N = 14$).

The adrenals were heavier on the left side of the body than on the right, when corrected for body weight (left adrenal: $M = 22.49 \pm .64$; right adrenal $M = 21.28 \pm .62$), $F(1, 59) = 7.58$, $p = .008$. The interaction of condition and body location was not statistically significant, $F(3, 59) = 0.14$, $p = .929$.

Among all species and sexes, the left adrenals were significantly heavier than those on the right side of the body (See Table 3-3 for complete means and analyses).

Testes Weight

Testes Weights were not significantly affected by the condition within any of the species, whether they were or were not corrected for differences in body weight. Complete means and analyses are located in Table 3-4. The only significant effects were attributed to small asymmetries in the left and right testis weights within meadow voles and montane voles (see means and effect of position in Table 3-4).

Seminal Vesicle Weight

The experimental conditions did not significantly influence the seminal vesicle weight within any species, whether they were or were not corrected for differences in body weight (see complete means and analyses in Table 3-5.

Ovarian Weight

The experimental conditions did not significantly influence the ovarian weight of any species, whether they were or were not corrected for differences in body weight (see means and analyses in Table 3-6).

Uterine Weight

The experimental condition did not significantly influence the uterine weights among the pine voles, prairie voles, or meadow voles (see means and analyses for all species in Table 3-7). However, significant differences were evident in the uterine weights of the montane voles as a result of the condition.

Montane voles. The condition significantly influenced the uterine weights of montane voles (see Figure 3-3). When uterine weights were compared, without correcting for differences in body weight, the uteri of females in the Control condition (M = 27.46 $\pm$ 1.92) weighed significantly more than the uteri found in the Family condition (M = 18.56 $\pm$ 1.92) and in the Female condition (M = 16.52 $\pm$ 2.22) (main effect of condition, $F(3, 60) = 4.55$, $p = .006$). The mean uterine weight among those in the Male condition was intermediate to those in the other conditions (M = 24.35 $\pm$ 2.97) and was not significantly different from them. This result is the first example, within a controlled environment, of sexual suppression in female montane voles that are exposed to the bedding from a family group or from adult females.

When the analysis of uterine weight was corrected for differences in body weight among montane voles, the results were similar to those of the unadjusted analysis (main effect of condition, $F(3, 60) = 4.61$, $p = .006$ (see Figure 3-3). The adjusted uterine weights were significantly greater in the Control condition (M = 97.71 $\pm$ 10.65) than in the Family condition (M = 61.02 $\pm$ 4.42) or Female condition (M = 58.92 $\pm$ 5.74). However, in addition, the females of the Male condition (M = 91.21 $\pm$ 14.45) had significantly heavier uteri than those in the Family condition.

Status of Vaginal Perforation

The total numbers and percentages of females that became vaginally perforate during the course of the study are summarized in Table 3-8 for all species. Clear differences were evident among the species in their modal pattern of vaginal perforation. Pine voles displayed the most atypical pattern of vaginal perforation with respect to the other species. None of the 66 pine voles were perforate at the beginning of the study (week 3) and only 2 of the 66 (3.0%) became perforate during the 9-week study. This slight shift in the frequency of pine voles that became perforate was not significant between weeks 3 and 9 (McNemar chi-square: $X^2 = .50$, $p < .479$). Nearly all of the females of the other species became perforate at some time during the study (see Table 3-8).

The percentages of females that were perforate at the beginning of the test were different among the four species, $X^2(3, N = 258) = 70.08$, $p < .001$. Post-hoc comparisons (chi-square) revealed that all species differed significantly from pine voles in the proportions of females that were perforate on week 3. However, significantly fewer prairie voles were perforate at week 3 than were meadow voles, ($X^2(1, N = 167) = 18.36$, $p < .001$, and montane voles, $X^2(1, N = 161) = 12.18$, $p < .001$. Meadow voles and montane voles did not differ significantly on this measure, $X^2(3, N = 186) = .91$, $p < .341$.

Delay until Vaginal Perforation

Statistical analyses of the delays of vaginal perforation were problematic, because properly they would be limited to those females that were not perforate on the first day of the study (week 3). However, the majority of all meadow voles (34 of 62 or 55%) and many montane voles (26 of 64 or 41%) were perforate on the initial day of the study and would be excluded from the analysis. In contrast, only 5 of 66 (8%) prairie voles and none of the 66 pine voles were perforate on the first day of the study. The total number of subjects and percentages of subjects for which vaginal smears were obtained are summarized in Tables 3-9 and 3-10 for each species and by condition.

The species differed appreciably in the time at which they became vaginally perforate. Figure 3-4 displays the cumulative percentages of females that became perforate throughout the study as a function of species. Nearly 80% of the meadow voles became perforate within the first 4 weeks of age, while prairie voles reached the 80% mark nearly a week later (Day 33). Over 50% of the montane voles were perforate by day 23, but the cumulative percentage of all montane voles did not reach the 80% criterion until day 41. Only two pine voles became perforate, the first at 44 days of age and the second on last day of the study (Day 63).

The mean ages (in days) that vaginal perforation occurred for subjects in each species and condition, that

were imperforate on the first day of the study, are shown in Figure 3-5. Sample sizes were not uniform across all species and conditions because of the different patterns of typical vaginal opening. Total numbers of subjects ranged from 14-16 for prairie voles, 5-6 for meadow voles, and 8-10 for montane voles among the conditions.

The condition was not found to significantly influence perforation latency among the prairie voles, (Kruskal-Wallis ANOVA, prairie voles: H (3, N = 59) = 4.34, p = .23) or montane voles: H (3, N = 36) = 5.74, p = .12). Meadow voles were not analyzed statistically because of the small number of subjects amenable to this analysis (N = 22).

Vaginal Smears

Three types of cells were classified and counted from the vaginal smears, following the method of Taylor (1990/1991). The cell types analyzed included cornified cells, nucleated cells, and leukocytes. Frequencies of each type were converted to percentages of the total number of cells in each smear, because of the variability in the total number of cells obtained per smear. Similar conversions of cell frequencies have been used previously in studies with laboratory rats (e.g., McClintock, 1983, 1984) and with voles (e.g., Sawrey, 1989/1990; Shapiro & Dewsbury, 1990). Data from female pine voles were excluded from the analysis because only two became perforate during the study.

All species produced vaginal smears that were typically dominated by either cornified cells or leukocytes, with

appreciably fewer nucleated cells. These characteristics of smears were typical of those found previously for several species of voles (e.g., Sawrey & Dewsbury, 1985; Taylor, 1990/1991). Nucleated cells were relatively few in number and relatively constant in proportion throughout the duration of the study. In contrast, cornified cells and leukocytes were typically in reverse proportion to one another when changes in the percentages of cells were detected.

Prior to conducting statistical analyses, all cell percentages were pooled for each subject by two-day block intervals. This procedure generally reduced daily variability in the cell percentages across successive days. Prior to pooling, graphs were made of the data as a function of each day; no indications of cyclical fluctuations were evident.

Effect of condition: within-species comparisons of cell types. Two types of analysis were conducted for within-species comparisons of cell types. The first analysis consisted of comparing the proportions of each cell type as a function of the experimental conditions for each two-day block (Kruskal-Wallis ANOVA by Ranks tests). Thus, a total of 21 two-day blocks (representing Days 21-22 through 61-62) were analyzed sequentially to determine if the condition influenced the percentages of cell types. In the case of a significant result, post-hoc comparisons were

made to identify which conditions differed significantly (Mann-Whitney U Tests).

In the second analysis, repeated-measures analyses were used to assess whether significant changes in the percentages of each cell type occurred as a function of age. These analyses were done separately for each condition and species. Cell percentages were analyzed on alternate blocks of days representing the ages of 33-34 days through 61-62 days (Friedman ANOVA by ranks tests). This reduced sample of days was necessary due to fewer smears available during the youngest ages and thus the necessary exclusion of data from repeated-measures analysis (see Table 3-9). Thus, a series of eight means was compared in each repeated-measures analysis. Post-hoc comparisons (Wilcoxon Matched Pairs Tests) were used to identify where significant changes occurred across the blocks of days when the Friedman ANOVA was found to be statistically significant (p's $< .05$).

Prairie voles: cornified cells: effect of condition. The percentages of cornified cells from prairie voles are shown as a function of experimental condition in Figure 3-6 (each mean plotted represents the pooled data of individuals by two-day block intervals). It was evident that relatively little variation occurred in the cell percentages as a function of the condition among prairie voles. A statistically significant change was identified for only one of the two-day blocks (Day 27-28: $H(3, N = 35) = 8.90$, $p = .031$). During this block, subjects in the Female

condition had significantly more cornified cells than those in the Male condition (M's = 27.5% versus 17.9%; total numbers of subjects can be determined from Table 3-9).

Prairie voles: effect of age. Relatively small changes in the percentages of cornified cells occurred as a function of age. Repeated-measures analyses failed to detect any significant change in the cell percentages for subjects in any of the four conditions.

Additional comparisons of the percentages of nucleated cells and leukocytes have been placed in Appendix C. In general, the proportions of nucleated cells varied little as a function of the condition or age for all species. Thus, the proportions of leukocytes often varied inversely to the proportions of cornified cells and are somewhat redundant for purposes of analysis.

Meadow voles: cornified cells: effect of condition. The mean percentages of cornified cells as a function of the experimental condition are shown in Figure 3-7. Despite somewhat greater differences among the cell means than those found in prairie voles, and relatively large sample sizes (range of total N's = 47-56), no significant differences were found within any of the blocks of days as a function of condition. The lack of significant differences also held true for all comparisons of nucleated cells and leukocytes (Appendix C).

Meadow voles: effect of age. Repeated measures analyses revealed significant changes in the percentages of

cornified cells within all four conditions of meadow voles. There were general increases in the percentages of cornified cells for subjects in all conditions, although the relative percentages of cornified cells for the Control condition were typically the smallest among those from all conditions (see Figure 3-7; Control condition: $X^2(7, \underline{N} = 11) = 31.39$, $\underline{p} < .001$). Significant differences occurred among the following blocks of days for subjects in the Control condition (Note: differences indicated below are in the direction of later days being significantly higher in cell percentages than earlier days except where noted "*"): Days 33-34 versus Days 49-50 through Days 61-62; Days 37-38 versus Days 41-52, 49-50 through Days 61-62; Days 45-46 versus Days 53-54 through Days 61-62; Days 49-50 versus Days 57-58 through Days 61-62.

Significant changes in the percentages of cornified cells for subjects in the Family condition were evident, $X^2(7, \underline{N} = 11) = 29.85$, $\underline{p} < .001$), and were as follows: Days 33-34 versus Days 45-46 through Days 57-58; Days 37-38 versus Days 45-46 through Days 61-62; Days 41-42 versus Days 45-46 through Days 61-62.

Significant changes in the percentages of cornified cells for subjects in the Male condition were present, $X^2(7, \underline{N} = 12) = 36.97$, $\underline{p} < .001$), were as follows: Days 33-34 versus Days 41-42, Days 49-50 through Days 61-62; Days 37-38 versus Days 41-42, Days 49-50 through Days 61-62; Days

41-42 versus Days 53-54 through Days 61-62; Days 45-46 versus Days 49-50 through Days 61-62.

Significant changes in the percentages of cornified cells for subjects in the Female condition were present, $X^2(7, N = 13) = 52.08, p < .001$), were as follows: Days 33-34 versus Days 41-42 through Days 61-62; Days 37-38 versus Days 45-46 through Days 61-62; Days 41-42 versus Days 45-46 through Days 61-62; Days 45-46 versus Days 53-54 through Days 61-62; Days 49-50 versus 57-58.

Montane voles: cornified cells. The mean percentages of cornified cells among montane voles in the various conditions varied more as a function of condition than the percentages observed in prairie voles and meadow voles (compare Figures 3-8 for montane voles to Figures 3-6, and 3-7). Typically, there were gradual increases in the percentages of cornified cells throughout the study for montane voles.

Montane voles: effect of condition. Statistical differences were found among subjects in the various conditions on blocks of days including Days 53-54, 57-58, and 61-62. Among the first and last of these three blocks, the subjects in the Male and Control conditions had significantly greater mean percentages of cornified cells than those in the Female and Family conditions. The intermediate block (Days 57-58) also differed in the same manner, except the difference between the Male and Family

condition failed to reach a significant level (see Figure 3-8 for clarification).

Montane voles: effect of age. Repeated measures analysis revealed significant shifts in the percentages of cornified cells within each of the four conditions. Montane voles in the Control condition had significant shifts in the proportions of cornified cells between the following blocks of days: Days 33-34 versus 41-42, 49-50 through 61-62; Days 37-38 versus 57-58 through 61-62; Days 41-42 versus 53-54 through 61-62; Days 45-46 versus 49-50 through 61-62; Days 49-50 versus 57-58 through 61-62.

Significant shifts in the percentages of cornified cells were found for those in the Family condition among the following blocks of days: Days 33-34 versus 49-50, 57-58 through 61-62; Days 37-38 versus 57-58 through 61-62; Days 41-42 versus 49-50 through 61-62; Days 45-46 versus 61-62; Days 49-50 versus 61-62; Days 53-54 versus 57-58.

Changes among blocks of days for subjects of the Male condition were statistically significant among the following blocks of days: Days 33-34 versus 53-54 through 61-62; Days 37-38 versus 53-54 through 61-62; Days 41-42 versus 49-50 through 61-62; Days 45-46 versus 53-54 through 61-62; and Days 49-50 versus 53-54, and 61-62.

Finally, significant shifts in the percentages of cornified cells were identified for subjects in the Female condition among the following blocks of days: Days 33-34 versus 41-42 through 49-50, and 57-58 through 61-62; Days

37-38 versus 49-50 through 61-62; Days 45-46 versus 61-62; Days 49-50 versus 61-62; Days 53-54 versus 61-62; and Days 57-58 versus 61-62.

Discussion

Prairie voles and pine voles were expected to show fundamentally different patterns of response from those of the meadow voles and montane voles. I predicted that the less social meadow voles and montane voles would show pronounced sexual advancement when exposed to the bedding of unfamiliar opposite-sexed individuals, and show signs of sexual suppression when exposed to same-sex odors. Animals exposed to clean bedding (Control condition) were expected to have intermediate indices of sexual development compared to those in the other conditions. In contrast, I believed there would be little or no differences in sexual development within the prairie voles or pine voles.

The experimental results of the exposure to the soiled bedding on general and sexual development are discussed below for each of the measures.

Body Weight

Male pine voles were the only group that differed significantly in body weight as a function of the condition. Males in the Family and Male conditions weighed more than those in the Control and Female conditions (Figure 3-1). These results, if repeatable, are contrary to predictions. The results suggest that the olfactory cues associated with adult males or family groups cause general increases in the

rate of growth of males exposed to them, when compared to patterns of growth of males exposed to clean bedding or to odors from adult females. Unfortunately, it is not possible to compare these results with those of other studies, as pine voles have not been investigated in any similar manner.

However, general characteristics of natural populations of pine voles suggest that male pine voles are not sexually suppressed when living within family groups and often are in the presence of at least one other scrotal male (FitzGerald & Madison, 1983). Thus, limited information on the free-ranging behavior of pine voles is at least compatible with the notion that the exposure of males to the odors from family groups and/or other males in some manner contributes to increased body weight. One possible mechanism by which males might experience increased body weight is simply through increased food intake. Such a mechanism has been identified that led to increased weight gain in male musk shrews (Suncus murinus) when exposed to females (Wayne & Rissman, 1990).

It is not known why pine voles were the only species to experience differences in body weight as a function of the condition. Other studies have shown that the body weights of other species of Microtus can be affected by exposure to pheromonal cues. Female meadow voles have been shown to have significantly greater increases in body weight, compared to controls, when the urine of mature males trickled directly into the cages of females via small tubes

(Baddaloo & Clulow, 1981). Other research has provided evidence that male and female prairie voles could differ in body weight, by nearly 10 g respectively, as a function of whether they had separate air supplies or shared a common air supply within the colony (Batzli et al. 1977).

Together, the results of previous studies suggest that differences in body weight might have been detected within some of the other species, had the procedures been different. The use of a more continuous supply of urine and pheromones or a separate supply of air for each subject may have led to detectable differences as a function of the condition. However, given the generally small differences in body weights among the male pine voles and number of comparisons made, one must also consider the results were due to chance alone and not due to pheromonal differences. Additional study seems warranted to clarify whether these patterns of weight gain among male pine voles can be replicated, what mechanism accounts for differences in body weight, and what functional differences they may be producing.

Anogenital Distance

The anogenital distances among montane voles might have differed as a function of the conditions, but the results are ambiguous. Whereas the overall *F* statistic was statistically significant, post-hoc comparisons failed to detect significant differences among the groups. Males in the Control condition had a larger mean anogenital distance

than those in the Family condition, although it was not significantly different (p = .09; Neuman-Keuls test). This difference is in the hypothesized direction, but the absolute difference between the groups limits making any firm conclusion.

In an attempt to determine statistically whether the differences in anogenital distances among montane voles were systematic effects caused by the conditions, an analysis of covariance was conducted, using body weight at week 3 as a covariate. Results were similar to those of the primary analysis, i.e., the interaction of anogenital distance and week of measurement was statistically significant, $F(18, 360) = 1.84$, $p = 0.019$, although no post-hoc comparisons were significantly different. Thus, the results of the anogenital distance comparisons are only suggestive of differences produced by exposure to the different bedding. Unfortunately, no similar studies have been conducted with montane voles, or other species of *Microtus*, in order to make any comparisons between studies.

It is not known what functional difference, if any, a larger anogenital distance would reflect. Presumably, the measure reflects, in part, the relative size of the testes which lie proximally between the penis and the anus. In the present study, the correlation between testes weight and anogenital distance was relatively small among all montane voles (N = 64; r_s = .31), although it was slightly higher in the Control males alone (N = 16; r_s = .37). Thus,

differences in testis weight do not lend much support for the possibility that the differences in anogenital distance largely reflect differences in testis weight. The analysis of the testes weights of the montane voles did not reveal any significant influence of the conditions (see below). Additional study would seem necessary to establish whether the differences in anogenital distance in montane voles are repeatable outcomes of exposure to these substrates.

Adrenal Weight

The adrenals differed systematically in weight as a function of the treatment among male pine voles. The adrenals, when corrected for differences in body weight, from males in the Female condition were significantly heavier than those in the Control and Family conditions. Because males of the Female condition were significantly lighter in body weight than those in the Family and Male conditions, it is possible there is a causal relationship between increased adrenal activity and decreased body weight. Prior studies with male meadow voles have shown that subcutaneous injections of ACTH produced significant reductions in body weight and concurrent increases in adrenal weight than among control males (Pasley & Christian, 1971).

It is not known what differences there are in bedding soiled from male and female pine voles. Recent chemical analysis of volatile compounds in male and female pine vole urine did not reveal any qualitative difference in the

urinary profiles between them (Boyer et al. 1989). However, female urine contained three volatiles in higher concentrations than in male urine, whereas male urine contained one compound present in higher concentration than in female urine. Boyer et al. (1989) suggested there may be differences in the nonvolatile urinary fraction that might be investigated. Among house mice, the acceleratory pheromone produced by male house mice appears to be mediated by a nonvolatile urinary fraction contained in the urine (Vandenbergh et al. 1975; 1976).

Among all species, the adrenals from the left side of the body were significantly heavier than those located on the right. This difference in asymmetry may be common to all species of Microtus. Pinter (1968) reported that the adrenals in montane voles were larger on the left than on the right side of the body, regardless of the sex, age, or regime of diet or photoperiod. A similar asymmetry in adrenal weight had been reported for California voles (M. californicus)(Mullen, 1960). The specific functional difference of such asymmetry, if any, is not known.

Testes Weight

Comparisons of the testes weights did not indicate any effect of the experimental condition among any of the species. It is possible that changes in testicular weight among male Microtus are relatively unaffected by exposure to different pheromones, although exposure to stressful environments may produce substantial changes. Lecyk (1967)

provided evidence that male common voles (M. arvalis), that were housed adjacent to cages with crowded and sexually active voles, had lighter testes than control males. Other indirect evidence that testicular weight can change as a function of stress was provided by Pasley and Christian (1971). Male meadow voles, injected with ACTH across a series of days, had significant decreases in testes weights when compared to noninjected controls.

It is possible that testis weight is a relatively insensitive measure of male reproductive activity, at least when investigating the influence of pheromones or other olfactory stimuli on reproductive maturation. More precise measures of reproductive activity might reveal systematic differences among voles exposed to different pheromones. Measures such as the level of circulating androgen or characterization and counts of spermatozoa may be appropriate. Research with California voles has shown that titers of androgens were found to be significantly lower in males exposed to bedding from their mothers than in those exposed to bedding from unrelated males; testes weights were not found to differ among these groups (Rissman et al. 1984).

Seminal Vesicle Weight

The experimental condition did not have a noticeable effect on the seminal vesicle weight among any of the species. Why no significant differences were found is puzzling. Prior studies with California voles have shown

that differences in seminal vesicle weight can be found when males are exposed to different types of soiled bedding. The seminal vesicles of California voles that were exposed to bedding soiled by their own mother, their family group (including the sire), or an unrelated mother were significantly smaller than those of males reared in clean bedding (Rissman et al. 1984; Rissman & Johnston, 1985). In contrast, the presentation of soiled bedding from unfamiliar adult males, or bedding from the family that had been supplemented with bedding from the separated father, produced seminal vesicles of similar weight in both groups. Thus, the data from California voles suggest that the development of the seminal vesicles can be delayed in young males when they are exposed to odors from their mothers or from unrelated mothers, but not delayed by exposure to odors from unrelated adult males or from their fathers (Rissman et al. 1984).

Ovarian Weight

The experimental condition did not have a noticeable effect on the ovarian weight in any species, whether they were corrected or uncorrected for differences in body weight. However, previous studies suggest that the ovarian weight among some species of Microtus can be affected by exposure to pheromonal stimuli. Baddaloo and Clulow (1981) found that female meadow voles that were exposed to male urine, that had been designed to drip into the adjoining

(empty) half of divided cages, had significantly heavier ovaries than compared to those of control females.

Female pine voles have also been shown to have increases in ovarian development when exposed to pheromonal stimuli. Lepri and Vandenbergh (1986) placed individual females directly below the cages of animals of different stimulus qualities. This procedure enabled feces and urine from the animal(s) in the top cages to fall into the cages housing the females. Results indicated that the ovaries of the females exposed to the excreta from either individually housed intact males or castrated males were significantly heavier those of females exposed to an empty cage or to the excreta from singly-housed females or group-housed females.

Uterine Weight

By at least one standard, the uterus is considered to be the best and most reliable bioassay for circulating estrogens in female house mice (Bronson & Stetson, 1973). Female montane voles in the Control condition were found to have significantly heavier uteri than those in the Family and Female conditions (Figure 3-3). In addition, the uteri from those in the Control and Male conditions were also significantly heavier than those in the Family and Female conditions when the uteri were corrected for differences in body weight. These results are the first to demonstrate sexual suppression of female montane voles when they are exposed to adult female bedding or family bedding under controlled conditions. Previous field evidence had only

suggested such a phenomenon (Jannett, 1978). The results are in general agreement with predictions and complement the recent finding of sexual acceleration in female montane voles when they were exposed to male stimuli (Sawrey & Dewsbury, 1991). However, if the responses of uterine weight to the pheromones conform to the distinction of socially-dependent versus independent dichotomy, it is not clear why meadow voles also did not show differences in uterine weight when they were exposed to the different bedding types.

It is possible that many species of Microtus, including meadow voles, may require both direct exposure to males and their pheromones for complete reproductive activation. This type of synergy has been demonstrated in house mice (Drickamer, 1974; Bronson & Maruniak, 1975), montane voles (Sawrey & Dewsbury, 1991), prairie voles (Carter et al. 1987), and pine voles (Lepri & Vandenbergh, 1986), although the specific causes for it are not yet understood. For example, among female prairie voles, direct contact with male urine or housing them in male-soiled cages results in increased uterine weights, but typically does not elicit behavioral estrus (Carter et al. 1987). Thus, it is possible that female meadow voles may require more direct male stimulation in comparison to montane voles. Evidence found by Taylor (1990/1991) supports this hypothesis, as nulliparous female meadow voles showed extremely low copulation rates after being kept behind a wire-mesh barrier

for up to seven days with only 1 h of daily direct interactions with males.

Characteristics of Vaginal Perforation

Although none of the species differed significantly in the latency to become vaginally perforate as a function of exposure to soiled bedding, clear species differences were evident in the latencies of the species to become perforate (Figure 3-4). Meadow voles and montane voles became perforate either before or shortly after weaning (Day 21). In contrast, prairie voles were somewhat delayed in becoming perforate. Pine voles were even more extreme, with only two of the animals becoming perforate during the course of the study.

The few pine voles that become vaginally perforate during the course of the study do not seem uncharacteristic from personal observations and from other reports (e.g., Schadler & Butterstein, 1979). It appears that most female pine voles, whether housed in isolation or with siblings, do not become perforate until they are placed with an unfamiliar male or reach a considerably advanced age. In pilot work for the study presented, 10 adult female pine voles were examined for vaginal perforation. Whether pine voles were housed singly, or with other siblings, none was perforate despite ranging in age between 90 to 120 days.

Earlier work also supports the finding of substantial delays in reproductive activity among pine voles. Schadler and Butterstein (1979) found that among female pine voles

that had been paired for breeding with fertility tested males, the mean age of first conception for the females was 105 days. Thus, it appears that direct and relatively long exposure to males may be a normal prerequisite for vaginal perforation and reproductive activation in pine voles, even if they have reached a considerably advanced age. Substantial delays among female prairie voles to become vaginally perforate have also been documented. Richmond & Conaway (1969b) found that 98% of more than 200 individually housed females, or those in compatible female groups, remained in a state of persistent anestrus with imperforate vaginas between 3 to 5 weeks of age.

Within-species analysis of the perforation latencies among prairie voles did not suggest they are strongly affected by the olfactory cues. The overall comparison test was not statistically significant, despite there being a relatively large number of imperforate female prairie voles at the beginning of the study (N's = 14-16 per condition). Female prairie voles in the Male condition became perforate, on average, earlier than those in the Control condition (M's = 33.0 versus 27.5 days)(Figure 3-5).

One of the difficulties with analyzing the conditions' effect upon perforation latencies among meadow voles and montane voles was that many females were vaginally perforate at the beginning of the test and thus excluded from the formal analysis. Employing an earlier day of weaning did not seem plausible for all species, although it was

attempted in pilot work. Weaning pine voles at an earlier age than 21 days often resulted in their deaths within a day or two following separation. In retrospect, it would seem possible to wean each species at different ages that corresponded more closely to their species-typical age for weaning. Indices such as the cessation of nursing or the timing of the first day to ingest solid food is typically earlier for meadow voles and montane voles than they are for pine voles and prairie voles (McGuire & Novak, 1984; 1986). By weaning each species at an age that appears most appropriate for each species, it would be possible to test more readily how stimuli affected certain reproductive responses, at least within each species.

Vaginal Cytology

It was expected that the less social species would show higher proportions of cornified cells when the females were exposed to male bedding than would females exposed to bedding in the other conditions. Those in the Family and Female conditions were predicted to have fewer cornified cells than those in the Control condition. The patterns of cornified cells for all species that became vaginally perforate are shown in Figure 3-6 through Figure 3-8.

The percentages of cornified cells for prairie voles among the various conditions were similar, and statistically only one of the two-day blocks revealed a significant difference among conditions. It seems plausible that this one significant difference was spurious, because neither the

few preceding nor the few following blocks of days was statistically significant. In addition, the total number of multiple-comparisons tests was substantially high. This in turn would inflate the likelihood of detecting at least one statistically significant difference among the days compared. As predicted, female prairie voles revealed little response to the different bedding types.

Although variation in the cell percentages for meadow voles suggested an influence of condition, none were detected statistically (Figure 3-7). Inspection of the percentages of cornified cells suggests a trend of more cornified cells in females exposed to adult female odors than those receiving no odors (Control). A priori, the direction of this relationship was not expected but suggests further study because it is possible that meadow voles experience sexual acceleration when exposed to odors or pheromones of other adult females. Among house mice, urine from pregnant and lactating females accelerates sexual development in female mice (Drickamer & Hoover, 1979), as does the urine from female mice that are in estrus (Drickamer 1982c). It seems plausible that among meadow voles, pheromones contained in the urine of other meadow voles that are of either sex may cause sexual acceleration when compared to those that are not exposed to any pheromonal source, at least under certain conditions. Results from the analysis of vaginal smears of meadow voles

by Baddaloo and Clulow (1981) are in general agreement with this prediction.

There is reasonable evidence that the conditions affected the proportions of cornified cells of the montane voles, at least during the last 10 days of the study. The statistical results revealed systematic differences in the proportions of cells as a function of condition during the later portion of the study (Figure 3-8). Specifically, during three of the last five two-day blocks of smears, the females in the Male and Control conditions had substantially more cornified cells than those in the Family and Female conditions. Although there is the possibility that these relatively few significant results were due to chance, because of the relatively large number of comparison tests (21 for each species and condition representing Days 21 through 62), the distribution of the significant effects suggests a non-random pattern.

The differences among the uterine weights of the montane voles add additional support for the hypothesis that the percentages of cornified cells of montane voles reflect systematic differences as a function of the condition. The corrected uterine weights were significantly heavier from females in the Male and Control conditions compared to those from the Family and Female conditions. Thus, the percentages of cornified cells appear to reflect these differences; larger uteri were associated with higher percentages of cornified cells among the montane voles.

Given the present results, the montane voles appeared to be the species that was most affected by the exposure to the pheromones. The results of the current study and of earlier studies suggest that, even with similar levels of male exposure, prairie voles and pine voles take appreciably longer to become sexually receptive than montane voles and meadow voles, and typically require direct exposure to males for complete reproductive activation to occur (Carter et al. 1987; Lepri & Vandenbergh, 1986). Thus, results of the vaginal smear data provide limited support for the socially-dependent and socially-independent dichotomy. The less social species, meadow voles and montane voles, appear to be more sensitive to conspecific pheromonal cues than are the more social species. Additional study of these species, using common procedures, may be useful to determine if these results are valid, and to shed light on possible functional differences between the species.

Conclusions (Experiment 1)

A major theme that broadly characterizes the results of Experiment 1 was the absence of an effect of exposure to the different bedding types among the species. These results were surprising, considering that a number of studies have shown that the reproductive physiology of several species of voles can be influenced by exposure to the same types of olfactory stimuli or pheromones that were used in the present experiment (see Puberty Modulation in Voles).

A few studies have shown specifically that the transfer of chemosignals contained within soiled bedding can effectively produce changes in reproductive activity in house mice (Drickamer, 1982c) and in some species of voles. Lepri and Vandenbergh (1986) showed that exposure to chemosignals contained within the soiled bedding of family groups caused reproductive suppression among female pine voles. Female pine voles were paired with unfamiliar males and then housed in cages with either clean or family-soiled bedding for 48 h. Females that were housed on clean bedding had significantly heavier uteri and ovaries than females housed on family-soiled bedding.

Soiled bedding has also been shown to affect reproductive development among male California voles (Rissman et al. 1984). Males that were reared from weaning in bedding from their families had significantly lighter seminal vesicles when they were 45, 55, and 75 days of age than those reared in clean bedding (Rissman et al. 1984). Androgen levels were significantly higher among the males reared in clean bedding versus those reared on the family bedding on day 45. Together, these studies show that the method of exposing animals to soiled bedding can be an effective means to reveal specific changes in reproductive physiology, at least among some species of muroid rodents.

Several reasons can be proposed as possible explanations for the lack of substantial differences in most reproductive measures as a result of the different

conditions. It is possible that pheromones may be minimally involved, or their activity overestimated, with regulating the onset of reproduction in all or most species of Microtus. It is possible that pheromones are substantially more effective in regulating reproduction in other species, such as in house mice (e.g., Bronson, 1979; Vandenbergh & Coppola, 1986). Clearly more comparative research is needed to test this possibility. Seminatural studies may be an appropriate method in which to run biologically relevant experiments, while still enabling the close monitoring of reproductive development in individuals exposed to pheromonal sources.

If pheromones are critically involved with influencing the timing of reproduction among Microtus, there are at least three methodological reasons that could account for the relatively few differences found. First, it is possible that the pervasive odors within the colony rooms effectively eliminated many of the differences that might have been found had animals been reared in environments that controlled for all or most of the extraneous olfactory stimuli. All animals were reared and tested in colony rooms where the odors of other conspecifics animals were housed that varied in sex, age, and reproductive status, although the subjects had been on separate shelving for each condition. Some support for this hypothesis is found in the work of Batzli et al. (1977), who controlled the air supply to individual animals. Both male and female prairie voles

had substantially elevated gains in body weight across a three month period as compared to animals that were treated similarly but shared a common air supply with other prairie voles. In addition, Sawrey (1989/1990) found differences in the patterns of vaginal smears of female montane voles that had been housed either with six other females in a separate room or had been housed in a larger colony with animals of mixed sex and age. Vaginal smears revealed that the six separately housed females had smaller percentages of cornified cells than smears from females in the large colony room (11.9% and 29.8% respectively).

A second possible reason for the relatively few differences found among the conditions is the possibility that the daily handling procedure was stressful enough to effectively obscure or eliminate systematic differences. There is indirect evidence that suggests such a possibility. Olsen & Seabloom (1973) found that the event of captivity caused elevated and prolonged secretion of corticosterone in wild-caught meadow voles. It is known that increased secretion of adrenal corticoids, androgens, progesterone, and other steroids are associated with an inhibition of reproduction (e.g., Christian, 1975). Thus, the daily handling procedure may have been a significant source of stress that might have reduced differences in morphological and physiological growth between animals in the different conditions.

A third possible reason for the relatively few differences among the conditions stems from the experimental design. It is possible that larger differences would have been found among the conditions had the soiled bedding and pheromones been presented to the subjects more frequently than every other day. Some researches have used procedures whereby urine was channeled directly into the cages of subjects via tubes or they have placed cages with wire bottoms and urine donors directly over the subject's cage (e.g., Baddaloo & Clulow, 1981; Lepri & Vandenbergh, 1986); thus, a near-continuous supply of pheromonal odors had been available. Some of the information gathered in house mice suggests that some pheromones are relatively volatile and therefore short-lived in functional activity, while others appear to be more stable and active over a few days. The actual chemical composition and properties of the pheromones from voles remain largely unknown.

Future research that is designed to identify the effects of exposure to pheromones may be based on the general design of this study. However, three improvements in the design could be based on the three possible sources of largely negative results listed above. Ideally, subjects would have individual supplies of air, be minimally stressed through extraneously handling or exposure to other animals, and have a more continuous exposure to pheromonal sources. The apparatus of Baddaloo & Clulow (1981) represents a modification in procedure that might detect physiological

responses from subjects. Despite the few significant results found in the present study, the results of many other studies suggest that the comparative study of the functions of pheromones among species of Microtus, appears to be a productive area for further advances in the elaboration of their evolved reproductive and social patterns.

Table 3-1. Mean Body Weights (in g ± S.E.) at Week 9 and Analysis (Experiment 1).

	Condition				Analysis of Variance			
	Control	Family	Male	Female	Factor	df	F	p
Pine Voles: Males								
M =	21.24	22.71	22.96	20.64	Condition	3, 58	1.68	.181
	(0.53)	(0.88)	(1.05)	(0.65)	Week[a]	6, 348	600.87	<.001***
N =	17	16	14	16	Interaction	18, 348	1.98	.010**
Pine Voles: Females								
M =	18.87	21.40	20.88	20.45	Condition	3, 62	1.62	.192
	(0.64)	(1.03)	(0.73)	(0.80)	Week	6, 372	466.46	<.001***
N =	16	16	18	16	Interaction	18, 372	0.51	.953
Prairie Voles: Males								
M's =	31.94	32.42	31.74	30.90	Condition	3, 60	0.57	.631
	(1.41)	(1.28)	(1.46)	(1.35)	Week	6, 360	346.39	<.001***
N =	16	16	16	16	Interaction	18, 360	.35	.994
Prairie Voles: Females								
M =	26.65	26.58	27.91	26.78	Condition	3, 62	0.29	.830
	(1.03)	(0.74)	(1.31)	(.93)	Week	6, 372	247.61	<.001***
N =	18	16	16	16	Interaction	18, 372	0.36	.993

[a] Means for all weeks are located in Appendix A.
* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 3-1--continued. Mean Body Weights (in g ± S.E.) at Week 9 and Analysis (Experiment 1).

	Condition				Analysis of Variance			
	Control	Family	Male	Female	Factor	df	F	p
Meadow Voles: Males								
M =	39.18	41.64	43.49	43.13	Condition	3, 60	1.02	.390
	(2.14)	(1.97)	(1.28)	(1.58)	Week[a]	6, 360	589.35	<.001***
N =	16	16	16	16	Interaction	18, 360	1.55	.069
Meadow Voles: Females								
M =	30.95	30.65	32.02	32.79	Condition	3, 58	0.31	.818
	(1.30)	(1.34)	(1.14)	(1.55)	Week	6, 348	302.85	<.001***
N =	16	15	16	15	Interaction	18, 348	1.53	.075
Montane Voles: Males								
M =	37.70	37.22	39.12	38.64	Condition	3, 60	0.10	.954
	(1.67)	(1.51)	(2.04)	(1.35)	Week	6, 360	537.85	<.001***
N =	16	16	16	16	Interaction	18, 360	0.92	.545
Montane Voles: Females								
M =	28.92	30.16	28.37	27.67	Condition	3, 59	0.30	.818
	(1.43)	(1.71)	(1.43)	(1.44)	Week	6, 354	284.25	<.001***
N =	16	16	15	16	Interaction	18, 354	0.93	.532

[a] Means for all weeks are located in Appendix A.
* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 3-2. Mean Anogenital Distance (in mm ± S.E.) at Week 9 and Analysis (Experiment 1).

	Condition				Analysis of Variance			
	Control	Family	Male	Female	Factor	df	F	p
Pine Voles: Males								
M =	7.71	7.70	7.96	7.75	Condition	3, 58	1.07	.365
	(.22)	(.26)	(.25)	(.24)	Week[a]	6, 348	140.43	<.001***
N =	17	15	14	16	Interaction	18, 348	1.17	.282
Prairie Voles: Males								
M =	12.20	12.31	12.28	11.25	Condition	3, 59	1.36	.262
	(.38)	(.32)	(.35)	(.36)	Week	6, 354	125.71	<.001***
N =	15	16	16	16	Interaction	18, 354	.58	.907
Meadow Voles: Males								
M =	18.22	18.09	19.19	18.72	Condition	3, 60	0.41	.746
	(.61)	(.50)	(.55)	(.74)	Week	6, 360	241.53	<.001***
N =	16	16	16	16	Interaction	18, 360	0.95	.517
Montane Voles: Males								
M =	13.44	12.25	13.09	12.66	Condition	3, 60	.33	.798
	(.54)	(.31)	(.48)	(.36)	Week	6, 360	173.00	<.001***
N =	16	16	16	16	Interaction	18, 360	1.84	.019*

[a] Means for all weeks are located in Appendix B.
* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 3-3. Mean Adrenal Weights (± S.E.) and Analysis (Experiment 1).

	Condition				Analysis of Variance			
	Control	Family	Male	Female	Factor	df	F	p
Pine Voles: Males (mg tissue, uncorrected for differences in body weight)								
M =	4.68	4.56	4.89	5.22	Condition	3, 59	1.26	.296
Left	(.18)	(.36)	(.21)	(.17)	Position	1, 59	7.58	.008**
N =	17	16	14	16	Interaction	3, 59	0.17	.910
M =	4.43	4.42	4.59	4.91	(All Left M = 4.83 ± .12)			
Right	(.25)	(.26)	(.24)	(.24)	(All Right M = 4.59 ± .12)			
Pine Voles: Males (corrected for body weight; mg tissue/100 g body weight)								
M =	22.25	20.42	21.69	25.52	Condition	3, 59	3.32	.026*
Left	(1.03)	(1.62)	(1.06)	(.99)	Position	1, 59	7.58	.008**
N =	17	16	14	16	Interaction	3, 59	0.14	.929
M =	20.96	19.70	20.36	24.04	(All Left M = 22.49 ± .64)			
Right	(1.20)	(1.09)	(1.12)	(1.36)	(All Right M = 21.28 ± .62)			
Pine Voles: Females (mg tissue, uncorrected for differences in body weight)								
M =	4.61	4.24	4.68	4.35	Condition	3, 62	0.48	.696
Left	(.25)	(.31)	(.22)	(.26)	Position	1, 62	9.73	.002***
N =	16	15	18	16	Interaction	3, 62	0.27	.847
M =	4.14	4.01	4.32	4.10	(All Left M = 5.86 ± .20)			
Right	(.29)	(.25)	(.22)	(.31)	(All Right M = 5.26 ± .19)			

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 3-3--continued. Mean Adrenal Weights (± S.E.)and Analysis (Experiment 1).

	Condition				Analysis of Variance			
	Control	Family	Male	Female	Factor	df	F	p
Pine Voles: Females (corrected for body weight; mg tissue/100 g body weight)								
M =	24.66	20.03	22.62	21.50	Condition	3, 62	1.98	.126
Left	(1.47)	(1.28)	(1.02)	(1.29)	Position	1, 62	8.86	.004**
N =	16	15	18	16	Interaction	3, 62	0.28	.834
M =	22.19	18.97	21.10	20.10	(All Left M = 22.21 ± .65)			
Right	(1.66)	(.99)	(1.14)	(1.35)	(All Right M = 20.58 ± .65)			
Prairie Voles: Males (mg tissue, uncorrected for differences in body weight)								
M =	6.06	5.22	4.89	5.22	Condition	3, 60	2.62	.058
Left	(.42)	(.24)	(.19)	(.31)	Position	1, 60	121.01	<.001***
N =	16	16	16	16	Interaction	3, 60	2.36	.079
M =	5.19	4.27	4.34	4.65	(All Left M = 5.34 ± .16)			
Right	(.38)	(.16)	(.12)	(.30)	(All Right M = 4.61 ± .14)			
Prairie Voles: Males (corrected for body weight; mg tissue/100 g body weight)								
M =	19.10	16.32	15.83	16.97	Condition	3, 60	2.44	.073
Left	(1.09)	(.79)	(.92)	(.75)	Position	1, 60	135.70	<.001***
N =	16	16	16	16	Interaction	3, 60	2.44	.073
M =	16.32	13.48	14.15	15.15	(All Left M = 17.05 ± .46)			
Right	(.94)	(.69)	(.82)	(.81)	(All Right M = 14.77 ± .42)			

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 3-3--continued. Mean Adrenal Weights (± S.E.) and Analysis (Experiment 1).

Condition				Analysis of Variance			
Control	Family	Male	Female	Factor	df	F	p
Prairie Voles: Females (mg tissue, uncorrected for differences in body weight)							
M =	5.33	6.01	6.05	6.12	Condition	3, 62	1.21	.312
Left	(.29)	(.29)	(.42)	(.56)	Position	1, 62	83.22	<.001***
N =	18	16	16	16	Interaction	3, 62	0.98	.407
M =	4.61	5.36	5.62	5.54	(All Left M = 5.86 ± .20)			
Right	(.28)	(.32)	(.42)	(.49)	(All Right M = 5.26 ± .19)			
Prairie Voles: Females (corrected for body weight; mg tissue/100 g body weight)							
M =	20.18	22.68	21.98	22.87	Condition	3, 62	1.18	.323
Left	(.92)	(1.04)	(1.59)	(1.72)	Position	1, 62	86.82	<.001***
N =	18	16	16	16	Interaction	3, 62	0.98	.403
M =	17.40	20.21	20.33	20.72	(All Left M = 21.88 ± .66)			
Right	(.93)	(1.18)	(1.48)	(1.52)	(All Right M = 19.60 ± .65)			
Meadow Voles: Males (mg tissue, uncorrected for differences in body weight)							
M =	3.59	3.25	3.52	3.53	Condition	3, 60	0.74	.528
Left	(.29)	(.16)	(.15)	(.20)	Position	1, 60	17.62	<.001***
N =	17	16	16	16	Interaction	3, 60	0.24	.862
M =	3.30	2.91	3.17	3.03	(All Left M = 3.47 ± .11)			
Right	(.21)	(.18)	(.16)	(.16)	(All Right M = 3.11 ± .09)			

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 3-3--continued. Mean Adrenal Weights (± S.E.) and Analysis (Experiment 1).

	Condition				Analysis of Variance			
	Control	Family	Male	Female	Factor	df	F	p
Meadow Voles: Males (corrected for body weight; mg tissue/100 g body weight)								
M =	9.30	7.97	8.16	8.38	Condition	3, 60	1.46	.233
Left	(.83)	(.45)	(.37)	(.58)	Position	1, 60	14.45	<.001***
N =	17	16	16	16	Interaction	3, 60	0.23	.874
M =	8.71	7.18	7.39	7.29	(All Left M = 8.47 ± .30)			
Right	(.77)	(.49)	(.45)	(.46)	(All Right M = 7.67 ± .29)			
Meadow Voles: Females (mg tissue, uncorrected for differences in body weight)								
M =	7.94	8.15	8.12	8.27	Condition	3, 58	0.14	.935
Left	(.64)	(.74)	(.67)	(.43)	Position	1, 58	34.71	<.001***
N =	16	15	16	15	Interaction	3, 58	1.18	.321
M =	7.24	6.34	6.37	6.96	(All Left M = 8.12 ± .31)			
Right	(.55)	(.62)	(.59)	(.52)	(All Right M = 6.73 ± .28)			
Meadow Voles: Females (corrected for body weight; mg tissue/100 g body weight)								
M =	26.30	27.44	25.53	25.66	Condition	3, 58	0.29	.829
Left	(2.13)	(2.80)	(2.01)	(1.42)	Position	1, 58	32.11	<.001***
N =	16	15	16	15	Interaction	3, 58	1.12	.345
M =	23.81	21.20	19.88	21.74	(All Left M = 26.22 ± 1.05)			
Right	(1.76)	(2.20)	(1.75)	(1.85)	(All Right M = 21.67 ± .94)			

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 3-3--continued. Mean Adrenal Weights (± S.E.) and Analysis (Experiment 1).

Condition				Analysis of Variance			
Control	Family	Male	Female	Factor	df	F	p
Montane Voles: Males (mg tissue, uncorrected for differences in body weight)							
M =	2.91	3.01	2.81	2.74	Condition	3, 59	0.33	.799
Left	(.19)	(.19)	(.17)	(.16)	Position	1, 59	42.77	<.001***
N =	16	16	16	16	Interaction	3, 59	0.46	.709
M =	2.56	2.57	2.53	2.42	(All Left M = 2.87 ± .08)			
Right	(.14)	(.16)	(.11)	(.17)	(All Right M = 2.52 ± .07)			
Montane Voles: Males (corrected for body weight; mg tissue/100 g body weight)							
M =	7.85	8.32	7.47	7.27	Condition	3, 59	0.53	.665
Left	(.59)	(.68)	(.61)	(.44)	Position	1, 59	41.33	<.001***
N =	16	16	16	16	Interaction	3, 59	0.40	.749
M =	7.00	7.14	6.70	6.42	(All Left M = 7.73 ± .29)			
Right	(.51)	(.62)	(.43)	(.43)	(All Right M = 6.82 ± .25)			
Montane Voles: Females (mg tissue, uncorrected for differences in body weight)							
M =	6.49	6.26	6.02	5.67	Condition	3, 60	0.26	.850
Left	(.51)	(.48)	(.41)	(.33)	Position	1, 60	9.99	.002**
N =	16	17	15	16	Interaction	3, 60	1.08	.360
M =	5.86	5.56	5.55	5.64	(All Left M = 6.11 ± .22)			
Right	(.48)	(.45)	(.36)	(.52)	(All Right M = 5.65 ± .22)			

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 3-3--continued. Mean Adrenal Weights (± S.E.) and Analysis (Experiment 1).

	Condition				Analysis of Variance			
	Control	Family	Male	Female	Factor	df	F	p
Montane Voles: Females (corrected for body weight; mg tissue/100 g body weight)								
M = Left	23.55 (2.56)	20.96 (1.22)	21.92 (1.74)	21.05 (1.27)	Condition	3, 60	0.39	.758
					Position	1, 60	7.97	.006**
N =	16	17	15	16	Interaction	3, 60	1.06	.370
M = Right	21.11 (2.25)	18.51 (1.09)	20.25 (1.61)	21.11 (2.22)	(All Left M = 21.85 ± .87)			
					(All Right M = 20.22 ± .91)			

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 3-4. Mean Testes Weights (in mg ± S.E.) and Analysis (Experiment 1).

	Condition				Analysis of Variance			
	Control	Family	Male	Female	Factor	df	F	p
Pine Voles: (mg tissue, uncorrected for differences in body weight)								
M =	20.09	19.87	22.34	22.04	Condition	3, 59	0.75	.526
Left	(1.45)	(1.84)	(2.10)	(1.55)	Position	1, 59	0.003	.956
N =	17	16	14	16	Interaction	3, 59	0.41	.747
M =	19.91	19.46	22.34	22.09				
Right	(1.45)	(1.84)	(1.98)	(1.39)				
Pine Voles: (corrected for body weight; mg tissue/100 g body weight)								
M =	95.77	88.14	99.89	108.11	Condition	3, 59	1.38	.255
Left	(7.45)	(7.53)	(9.40)	(8.28)	Position	1, 59	0.02	.873
N =	17	16	14	16	Interaction	3, 59	0.48	.693
M =	94.87	86.81	99.87	111.28				
Right	(7.43)	(7.92)	(9.13)	(7.83)				
Prairie Voles: (mg tissue, uncorrected for differences in body weight)								
M =	147.26	140.01	162.18	139.50	Condition	3, 60	0.95	.421
Left	(14.70)	(8.09)	(10.98)	(10.17)	Position	1, 60	0.01	.891
N =	16	16	16	16	Interaction	3, 60	0.21	.882
M =	145.81	140.46	164.64	139.01				
Right	(15.23)	(8.29)	(12.57)	(10.55)				

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 3-4--continued. Mean Testes Weights (in mg ± S.E.) and Analysis (Experiment 1).

	Condition				Analysis of Variance			
	Control	Family	Male	Female	Factor	df	F	p
Prairie Voles: (corrected for body weight; mg tissue/100 g body weight)								
M =	467.77	429.71	505.93	447.20	Condition	3, 60	1.68	.179
Left	(42.22)	(16.54)	(25.33)	(20.55)	Position	1, 60	0.06	.801
N =	16	16	16	16	Interaction	3, 60	0.29	.831
M =	462.69	431.16	517.01	446.19				
Right	(42.80)	(17.19)	(22.40)	(19.95)				
Meadow Voles: (mg tissue, uncorrected for differences in body weight)								
M =	503.48	447.69	490.08	529.88	Condition	3, 61	0.85	.471
Left	(31.13)	(39.38)	(36.09)	(34.76)	Position	1, 61	13.87	<.001***
N =	17	16	16	16	Interaction	3, 61	1.93	.132
M =	505.27	466.74	496.57	545.57	(All Left M = 492.95 ± 17.63)			
Right	(32.73)	(42.09)	(38.06)	(31.84)	(All Right M = 503.56 ± 18.07)			
Meadow Voles: (corrected for body weight; mg tissue/100 g body weight)								
M =	1278.29	1109.82	1113.91	1238.01	Condition	3, 61	1.17	.326
Left	(54.28)	(93.86)	(72.00)	(79.97)	Position	1, 61	13.39	<.001***
N =	17	16	16	16	Interaction	3, 61	2.13	.105
M =	1282.24	1158.07	1127.58	1274.68	(All Left M = 1186.44 ± 38.16)			
Right	(56.28)	(99.57)	(74.95)	(72.13)	(All Right M = 1211.74 ± 38.43)			

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 3-4--continued. Mean Testes Weights (in mg ± S.E.) and Analysis (Experiment 1).

	Condition				Analysis of Variance			
	Control	Family	Male	Female	Factor	df	F	p
Montane Voles: (mg tissue, uncorrected for differences in body weight)								
M = Left	165.79 (8.08)	155.31 (8.06)	154.27 (8.11)	147.48 (5.03)	Condition	3, 60	1.06	.371
					Position	1, 60	4.15	.045*
N =	16	16	16	16	Interaction	3, 60	0.13	.938
M = Right	163.57 (7.50)	150.96 (8.33)	151.02 (9.14)	145.46 (4.54)	(All Left M = 155.71 ± 3.72)			
					(All Right M = 152.75 ± 3.79)			
Montane Voles: (corrected for body weight; mg tissue/100 g body weight)								
M = Left	451.39 (26.79)	427.33 (25.53)	403.81 (22.94)	390.55 (21.05)	Condition	3, 60	1.31	.276
					Position	1, 60	6.76	.011*
N =	16	16	16	16	Interaction	3, 60	0.12	.944
M = Right	445.17 (25.34)	415.73 (25.82)	394.03 (24.24)	383.03 (17.38)	(All Left M = 418.27 ± 12.15)			
					(All Right M = 409.49 ± 11.82)			

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 3-5. Mean Seminal Vesicle Weights (in mg ± S.E.) and Analysis (Experiment 1).

	Condition				Analysis of Variance			
	Control	Family	Male	Female	Factor	df	F	p
Pine Voles: (mg tissue, uncorrected for differences in body weight)								
M =	25.62	23.98	29.06	27.09	Condition	3, 59	0.26	.853
	(5.61)	(2.82)	(3.60)	(3.57)				
N =	17	16	14	16				
Pine Voles: (corrected for body weight; mg tissue/100 g body weight)								
M =	115.97	104.57	127.60	133.30	Condition	3, 59	0.54	.654
	(22.03)	(10.76)	(15.09)	(18.09)				
Prairie Voles: (mg tissue, uncorrected for differences in body weight)								
M =	211.02	187.22	193.06	161.76	Condition	3, 60	0.97	.408
	(23.62)	(16.16)	(23.72)	(17.66)				
N =	16	16	16	16				
Prairie Voles: (corrected for body weight; mg tissue/100 g body weight)								
M =	653.62	569.78	599.05	527.27	Condition	3, 60	0.90	.442
	(65.26)	(30.48)	(60.36)	(59.74)				

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 3-5--continued. Mean Seminal Vesicles (in mg ± S.E.) and Analysis (Experiment 1).

	Condition				Analysis of Variance			
	Control	Family	Male	Female	Factor	df	F	p
Meadow Voles: (mg tissue, uncorrected for differences in body weight)								
M =	269.46	281.26	317.44	362.02	Condition	3, 61	1.81	.153
	(28.96)	(32.58)	(32.18)	(30.07)				
N =	17	16	16	16				
Meadow Voles: (corrected for body weight; mg tissue/100 g body weight)								
M =	695.37	675.15	730.41	839.85	Condition	3, 61	1.14	.336
	(64.53)	(68.21)	(72.77)	(67.33)				
Montane Voles: (mg tissue, uncorrected for differences in body weight)								
M =	160.32	142.56	175.96	177.37	Condition	3, 60	0.83	.480
	(10.82)	(17.28)	(22.98)	(18.07)				
N =	16	16	16	16				
Montane Voles: (corrected for body weight; mg tissue/100 g body weight)								
M =	433.63	370.09	435.75	459.97	Condition	3, 60	0.94	.422
	(32.00)	(36.41)	(41.67)	(46.20)				

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 3-6. Mean Ovarian Weights (in mg ± S.E.) and Analysis (Experiment 1).

	Condition				Analysis of Variance			
	Control	Family	Male	Female	Factor	df	F	p
Pine Voles: (mg tissue, uncorrected for differences in body weight)								
M =	1.96	1.88	2.40	2.21	Condition	3, 61	1.56	.207
Left	(.12)	(.14)	(.18)	(.14)	Position	1, 61	0.007	.933
N =	16	15	18	16	Interaction	3, 61	2.15	.102
M =	2.12	1.91	2.11	2.28				
Right	(.16)	(.20)	(.13)	(.14)				
Pine Voles: (corrected for body weight; mg tissue/100 g body weight)								
M =	10.48	9.09	11.47	10.84	Condition	3, 61	1.92	.134
Left	(.65)	(.65)	(.80)	(.60)	Position	1, 61	0.001	.996
N =	16	15	18	16	Interaction	3, 61	1.75	.165
M =	11.31	9.24	10.16	11.16				
Right	(.81)	(.91)	(.64)	(.52)				
Prairie Voles: (mg tissue, uncorrected for differences in body weight)								
M =	2.43	2.96	2.71	2.59	Condition	3, 62	1.86	.144
Left	(.18)	(.26)	(.25)	(.15)	Position	1, 62	0.09	.757
N =	18	16	16	16	Interaction	3, 62	0.39	.755
M =	2.52	3.11	2.66	2.51				
Right	(.16)	(.22)	(.22)	(.15)				

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 3-6--continued. Mean Ovarian Weights (in mg ± S.E.) and Analysis (Experiment 1).

	Condition				Analysis of Variance			
	Control	Family	Male	Female	Factor	df	F	p
Prairie Voles: (corrected for body weight; mg tissue/100 g body weight)								
M =	9.27	11.26	9.65	9.94	Condition	3, 62	2.12	.106
Left	(.57)	(1.00)	(.73)	(.76)	Position	1, 62	0.08	.774
N =	18	16	16	16	Interaction	3, 62	0.26	.850
M =	9.28	11.83	9.66	9.72				
Right	(.47)	(.88)	(.83)	(.81)				
Meadow Voles: (mg tissue, uncorrected for differences in body weight)								
M =	5.37	5.67	6.76	6.67	Condition	3, 58	1.14	.338
Left	(.45)	(.59)	(.62)	(.33)	Position	1, 58	0.69	.409
N =	16	15	16	15	Interaction	3, 58	1.81	.155
M =	5.92	6.29	6.29	6.62				
Right	(.44)	(.46)	(.52)	(.30)				
Meadow Voles: (corrected for body weight; mg tissue/100 g body weight)								
M =	17.53	18.85	21.10	20.86	Condition	3, 58	0.55	.647
Left	(1.38)	(1.93)	(1.81)	(1.22)	Position	1, 58	0.85	.360
N =	16	15	16	15	Interaction	3, 58	1.71	.174
M =	19.46	20.56	19.75	20.86				
Right	(1.41)	(1.28)	(1.53)	(1.28)				

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 3-6--continued. Mean Ovarian Weights (in mg ± S.E.) and Analysis (Experiment 1).

	Condition				Analysis of Variance			
	Control	Family	Male	Female	Factor	df	F	p
Montane Voles: (mg tissue, uncorrected for differences in body weight)								
M =	2.89	2.89	2.94	2.24	Condition	3, 60	1.46	.233
Left	(.26)	(.21)	(.18)	(.16)	Position	1, 60	0.77	.381
N =	16	17	15	16	Interaction	3, 60	1.96	.129
M =	3.11	2.85	2.70	2.65				
Right	(.24)	(.23)	(.18)	(.26)				
Montane Voles: (corrected for body weight; mg tissue/100 g body weight)								
M =	10.05	9.75	10.46	8.19	Condition	3, 60	1.64	.189
Left	(.83)	(.62)	(.58)	(.45)	Position	1, 60	0.73	.393
N =	16	17	15	16	Interaction	3, 60	2.22	.094
M =	11.11	9.53	9.54	9.49				
Right	(.97)	(.53)	(.52)	(.66)				

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 3-7. Mean Uterine Weight (in mg ± S.E.) and Analysis (Experiment 1).

	Condition				Analysis of Variance			
	Control	Family	Male	Female	Factor	df	F	p
Pine Voles: (mg tissue, uncorrected for differences in body weight)								
M =	7.33	7.20	7.34	7.00	Condition	3, 61	0.05	.981
	(.86)	(.58)	(.60)	(.56)				
N =	16	15	18	16				
Pine Voles: (corrected for body weight; mg tissue/100 g body weight)								
M =	38.67	34.72	34.51	33.81	Condition	3, 61	0.69	.558
	(3.60)	(2.72)	(2.04)	(1.88)				
Prairie Voles: (mg tissue, uncorrected for differences in body weight)								
M =	11.19	13.59	13.92	12.16	Condition	3, 62	1.48	.228
	(.94)	(1.03)	(1.44)	(.71)				
N =	18	16	16	16				
Prairie Voles: (corrected for body weight; mg tissue/100 g body weight)								
M =	42.04	51.52	49.70	45.98	Condition	3, 62	1.38	.256
	(3.18)	(3.90)	(4.39)	(2.94)				

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 3-7--continued. Mean Uterine Weight (in mg ± S.E.) and Analysis (Experiment 1).

	Condition				Analysis of Variance			
	Control	Family	Male	Female	Factor	df	F	p
Meadow Voles: (mg tissue, uncorrected for differences in body weight)								
M =	22.57	25.17	29.49	25.04	Condition	3, 57	0.38	.767
	(3.83)	(5.11)	(6.05)	(3.32)				
N =	16	15	16	15				
Meadow Voles: (corrected for body weight; mg tissue/100 g body weight)								
M =	72.17	82.88	89.91	76.12	Condition	3, 57	0.29	.831
	(11.39)	(17.65)	(17.92)	(9.49)				
Montane Voles: (mg tissue, uncorrected for differences in body weight)								
M =	27.46	18.56	24.35	16.52	Condition	3, 60	4.55	.006**
	(2.39)	(1.92)	(2.97)	(2.22)				
N =	16	17	15	16				
Montane Voles: (corrected for body weight; mg tissue/100 g body weight)								
M =	97.71	61.02	91.21	58.92	Condition	3, 60	4.61	.005**
	(10.65)	(4.42)	(14.45)	(5.74)				

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 3-8. Numbers and Percentages of Vaginally Perforate Female Microtus (Experiment 2).

	Species			
	Pine	Prairie	Meadow	Montane
Total Number of Females in Study	66	66	62	64
Number of Females Perforate Week 3	0 (0.0%)	5 (7.6%)	34 (54.8%)	26 (40.6%)
Number of Females perforate by Week 9	2 (3.0%)	64 (97.0%)	56 (90.3%)	62 (96.9%)

Table 3-9. Species and Number of Subjects that were Vaginally Perforate on a given Day: (Experiment 1).

		Age in Days																				
		21	23	25	27	29	31	33	35	37	39	41	43	45	47	49	51	53	55	57	59	61
Condition		Prairie Voles																				
Control	N =	3	4	7	7	7	10	13	13	14	14	15	15	15	16	16	17	17	17	17	17	17
Family	N =	2	4	8	8	9	10	10	12	13	15	15	15	15	15	15	15	15	15	15	15	15
Male	N =	3	4	10	11	14	15	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16
Female	N =	3	5	8	9	12	13	15	15	16	16	16	16	16	16	16	16	16	16	16	16	16
Total	N =	11	17	33	35	42	48	54	56	59	61	62	62	62	63	63	64	64	64	64	64	64
		Meadow Voles																				
Control	N =	10	11	11	11	11	11	11	11	12	12	12	12	12	12	12	13	13	13	13	14	14
Family	N =	7	10	10	10	11	11	11	11	12	12	12	12	12	12	13	13	13	13	13	13	13
Male	N =	12	12	12	12	12	12	12	12	12	12	12	12	12	12	13	13	13	13	15	15	15
Female	N =	9	12	13	13	13	13	13	13	13	13	14	14	14	14	14	14	14	14	14	14	14
Total	N =	38	45	46	46	47	47	47	47	49	49	50	50	50	50	52	53	53	53	55	56	56
		Montane Voles																				
Control	N =	7	9	10	10	10	11	14	15	15	15	15	16	16	16	16	16	16	16	16	16	16
Family	N =	7	7	7	8	8	8	11	11	12	12	12	13	14	15	15	15	16	16	17	17	17
Male	N =	8	10	10	11	11	11	12	12	12	12	14	14	14	14	15	15	15	15	15	15	15
Female	N =	6	9	9	9	9	9	9	9	9	10	9	11	13	13	14	13	14	14	14	14	14
Total	N =	28	35	36	38	38	39	46	47	48	49	50	54	57	58	60	59	61	61	62	62	62

Table 3-10. Species and Percentages of Subjects that were Vaginally Perforate on a given Day (Experiment 1).

		Age in Days																			
		21	23	25	27	29	31	33	35	37	39	41	43	45	47	49	51	53	55	57	59	61
Condition																						
		Prairie Voles																				
Control	%	18	24	41	41	59	76	82	82	88	88	88	94	94	100	100	100	100	100	100	100	100
Family	%	13	27	53	53	60	67	67	80	87	100	100	100	100	100	100	100	100	100	100	100	100
Male	%	19	25	63	69	88	94	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Female	%	19	31	50	56	75	81	94	94	100	100	100	100	100	100	100	100	100	100	100	100	100
Total	%	17	27	52	55	66	75	84	88	92	95	97	97	97	98	98	100	100	100	100	100	100
		Meadow Voles																				
Control	%	71	79	79	79	79	79	79	79	86	86	86	86	86	86	86	93	93	93	93	100	100
Family	%	54	77	77	77	85	85	85	85	92	92	92	92	92	92	100	100	100	100	100	100	100
Male	%	80	80	80	80	80	80	80	80	80	80	80	80	80	80	87	87	87	87	100	100	100
Female	%	64	86	93	93	93	93	93	93	93	93	100	100	100	100	100	100	100	100	100	100	100
Total	%	68	80	82	82	84	84	84	84	88	88	89	89	89	89	93	95	95	95	98	100	100
		Montane Voles																				
Control	%	44	56	63	63	63	69	88	94	94	94	94	100	100	100	100	100	100	100	100	100	100
Family	%	41	41	41	47	47	47	65	65	71	71	71	76	82	88	88	88	94	94	100	100	100
Male	%	53	67	67	73	73	73	80	80	80	93	93	93	93	100	100	100	100	100	100	100	100
Female	%	43	64	64	64	64	64	64	64	64	71	64	79	93	100	93	100	100	100	100	100	100
Total	%	45	56	58	61	61	63	74	76	77	79	81	87	92	94	97	95	98	98	100	100	100

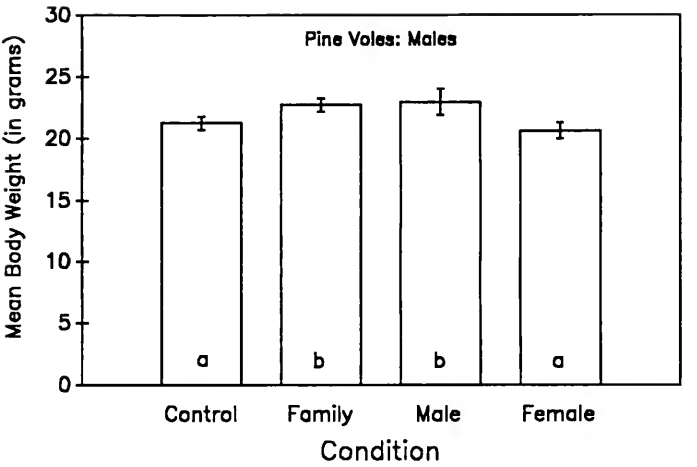

Figure 3-1. Mean body weights (in g ± standard error) of male pine voles in each condition at 9 weeks of age (see text for explanation of conditions). Columns with different letters are significantly different (p's < .05).

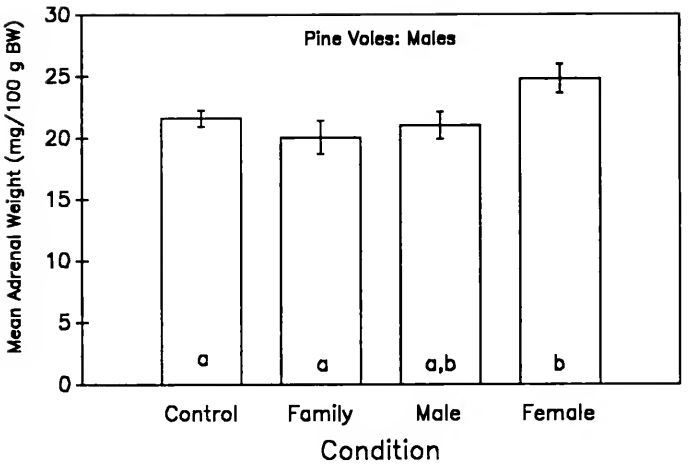

Figure 3-2. Mean Adrenal weights (corrected for differences in body weight; mg/100 g body weight ± standard error) of male pine voles in each condition at 9 weeks of age (see text for explanation of conditions). Columns with different letters are significantly different (*p*'s < .05).

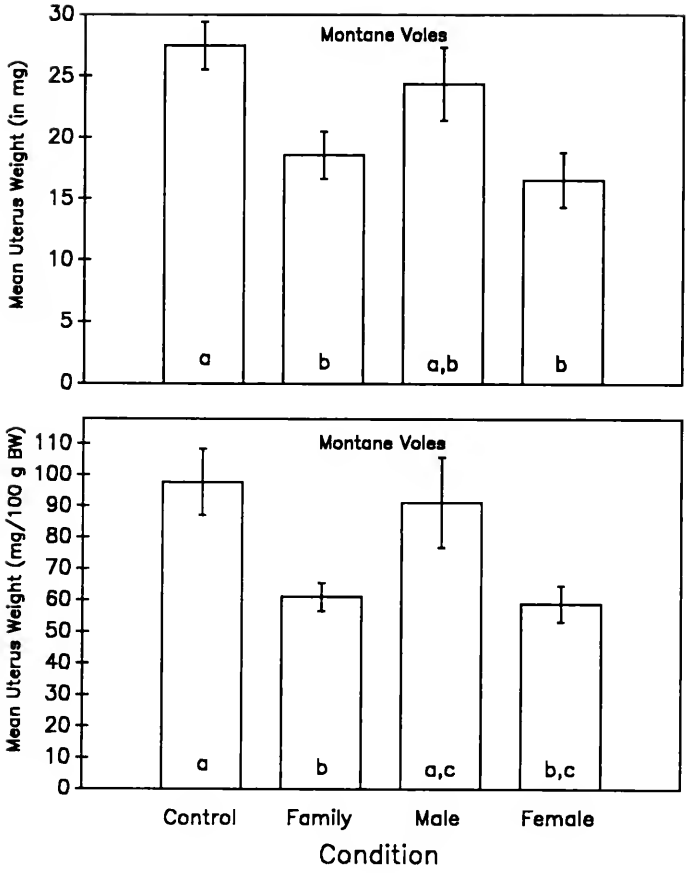

Figure 3-3. Mean Uterine weights (± standard error) of montane voles in each condition at 9 weeks of age (see text for explanation of conditions). a) uterine weight (uncorrected for differences in body weight) in mg; b) uterine weight corrected for differences in body weight (mg/100 g body weight). Columns with different letters are significantly different (*p*'s < .05).

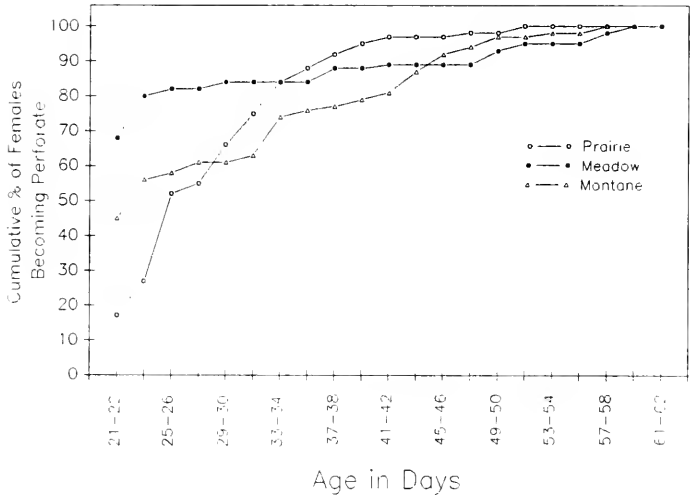

Figure 3-4. Cumulative percentages of females that became vaginally perforate during Experiment 1 at given ages (in days). Prairie: prairie voles; Meadow: meadow voles; Montane: montane voles.

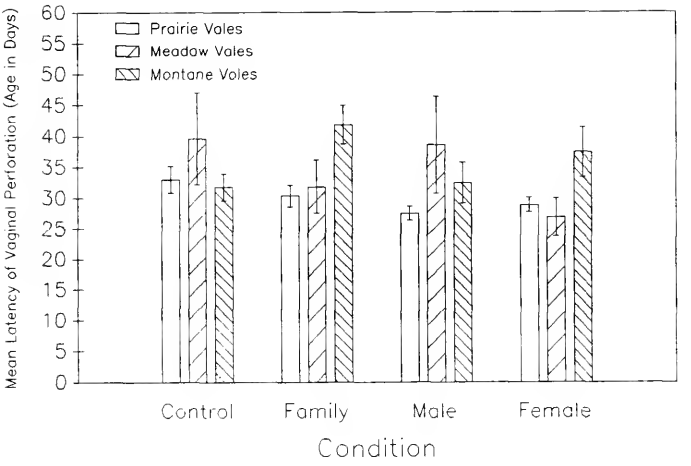

Figure 3-5. Mean age of vaginal perforation among Microtus (in days of age ± standard error) as a function of experimental condition. No significant differences were evident within each species (see text for explanation of experimental conditions).

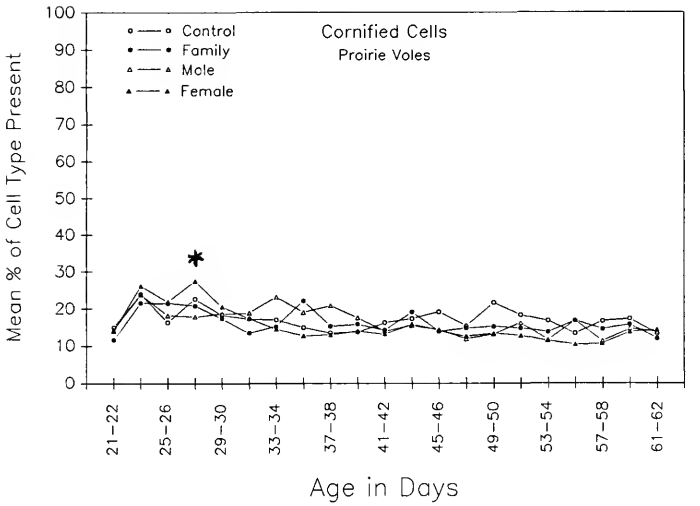

Figure 3-6. Mean percentages of cornified cells found in vaginal smears of prairie voles as a function of experimental condition and age (data are shown by two-day block intervals. "*" represents a significant differences (p's < .05) among conditions on given day (see text for explanation of conditions and significant differences).

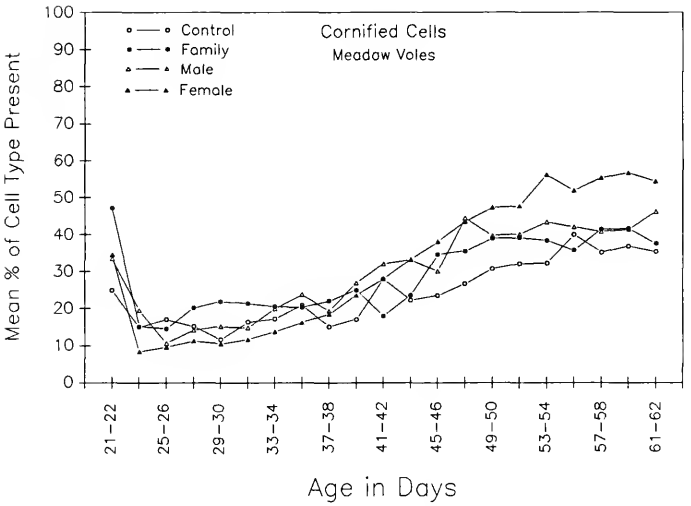

Figure 3-7. Mean percentages of cornified cells found in vaginal smears of meadow voles as a function of experimental condition and age (data are shown by two-day block intervals; see text for explanation of conditions).

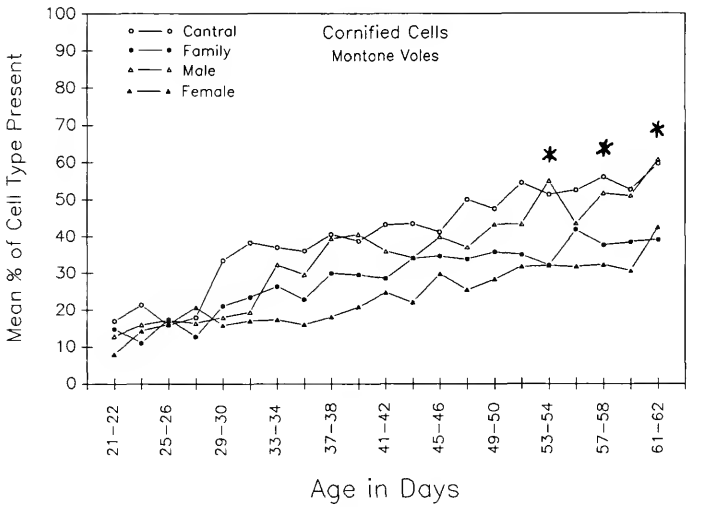

Figure 3-8. Mean percentages of cornified cells found in vaginal smears of montane voles as a function of experimental condition and age (data are shown by two-day block intervals. "*" represents significant differences (*p*'s < .05) among conditions on given day (see text for explanation of conditions and significant differences).

CHAPTER 4
BEHAVIORAL RESPONSES OF VOLES (MICROTUS) TO PUBERTY MODULATING STIMULI (EXPERIMENT 2)

Rationale

Although a considerable amount is known about the physiological responses of house mice and voles to pheromones that produce puberty modulation, very little is known about how these species respond behaviorally to their presence (see Coppola and O'Connell, 1988; Drickamer, 1989b). Knowledge about how individuals might behaviorally diminish or enhance the effects of pheromones found in the environment appear critical to understand the process of puberty modulation and possibly the formation of social and mating systems. Vandenbergh and Coppola (1986) suggested that we determine whether young animals will investigate or avoid urine that can influence puberty. It is necessary to determine whether behavior directed toward such stimuli can be influenced by the social environment or other relevant factors. For example, do female mice investigate the urine of males if more dominant females are present? Similarly, do fluctuations in resources, such as food availability, influence behavior and presumably the timing of puberty and reproduction?

Suggestive, although limited, data have been gathered for behavioral responses of mice to various cues. Female

mice will selectively avoid or approach puberty-modulating chemosignals depending upon their age and reproductive status. Drickamer (1989b) found that prepubertal female mice typically avoided odors of adult males, whereas postpubertal or adult females were preferentially attracted to them. Recording the behavioral responses of young male mice or voles to similar cues would be of interest, although no controlled studies have been reported.

In a related study, Drickamer (1988) provided evidence that early puberty and early reproduction were associated with a shorter life span for female mice compared with those that bred at later ages. Thus, it seems reasonable to assume that females will selectively avoid odors that cause them to attain puberty before their body systems can withstand the energetic demands of reproduction and hence limit their lifetime reproductive success. It can be predicted that young males would seek cues associated with females at a very early age, unless social factors inhibited them. Among house mice, aggression by territorial males toward other males is a pervasive phenomenon (Bronson & Coquelin, 1980).

Limited evidence suggests that investigation or avoidance of olfactory and pheromonal cues occurs among some species of voles, and may be influenced by the state of sexual maturity. Sawrey (1989/1990) conducted odor preference tests with adult male and female montane voles for male-soiled and female-soiled bedding. Females

preferred male-soiled bedding compared to female-soiled bedding. Somewhat surprisingly, male montane voles also preferred male-soiled bedding. Sawrey noted that although the preference of females for male bedding had been found in a variety of species, the result of the males' preference was unexpected. He suggested the possibility that the establishment of a territory exclusive of other males may be a prerequisite for the acquisition of mates by an unmated male. Additional data are needed to test this possibility. Similar measures gathered for other species of Microtus, and with subjects that varied in reproductive status, would be useful to determine how behavior might be functionally linked to puberty modulation and observed differences in social and mating systems.

There is suggestive evidence that voles avoid common sources of olfactory cues or pheromones in their natural environment. Wolff (1980) found that non-reproductive female taiga voles (M. xanthognathus) and subordinate males did not use scat piles produced by dominant males, although reproductively active females used them. It is possible to interpret this pattern as one of avoidance of male urinary cues, female cues, or both, by juveniles. Additional study is necessary to determine whether similar behavioral patterns occur in other species of Microtus and what function they serve.

The following study addresses the need for additional investigation of how behavior may modulate the timing of

puberty and how these behaviors may be related to the expressed social and mating system. Few data are available on such preferences; thus the experiment was largely exploratory, with no firm indications that changes in development would be associated with changes in olfactory preferences. Similar procedures were used for each sex and species to enable meaningful comparisons among them.

Measures and predictions. Because Experiments 1 and 2 were run concurrently, it was not possible to derive predictions based on the results of Experiment 1. However, I made general predictions that the behavioral patterns would reflect some of the patterns seen in Experiment 1. I predicted that those species that experienced substantial reproductive change from exposure to the stimuli in Experiment 1, would show related behavioral differences in Experiment 2. Specifically, because it was believed that the two less social species would show signs of puberty acceleration when exposed to opposite-sex stimuli in Experiment 1, I expected the meadow voles and montane voles would prefer opposite-sex odors and would either show no preference toward stimuli that caused puberty inhibition or reveal an aversion to them.

In addition to these predictions on odor preference, additional predictions were based on patterns of general development. Providing that the social species were found to be affected by the stimuli in Experiment 1, I believed that the more social and generally slower developing

species, pine voles and prairie voles, would not be attracted to odors of opposite-sexed adults or possibly be attracted to odors from same-sexed adults when they were very young (4 weeks of age). These predictions were based on Drickamer's (1989b) finding that prepubertal female mice typically avoided odors of adult males, while postpubertal or adult females were preferentially attracted to them. I believed that as the more social species reached maturity, there would be a shift in their behavior to one of a preference for opposite-sex odors.

In contrast to the highly social species, I believed that the less social species, meadow voles and montane voles, would display an earlier and sustained attraction for odors of the opposite sex, providing they had been affected by the stimuli in Experiment 1. These predictions were based on the generally quicker rates of maturation among meadow voles and montane voles compared to the other species (McGuire & Novak, 1984, 1986; Nadeau, 1985), and the demonstration of olfactory preferences for opposite-sex odors by meadow voles during periods of reproductive activation during the summer months (Ferkin & Seamon, 1987).

Two behavioral measures were recorded to investigate how behavior might be associated with changes in development and expressed differences in social and mating system. First, measures of odor preference were recorded by measuring the total duration subjects remained within 1 cm of each stimulus. Second, the total durations subjects

remained in the center (neutral) area of the test cage were recorded. The second measure was recorded to reveal possible differences in general aversion to both stimuli among the subjects. It was possible that subjects might not display olfactory preferences for one stimulus versus another, although differences could exist within or between species in general aversion to the stimuli that might be revealed by this measure.

Method

Subjects

A total of 129 animals, 15 to 18 males and 15 to 18 females from each of four species of voles served as subjects. Species included pine voles (Microtus pinetorum), prairie voles (M. ochrogaster), meadow voles (M. pennsylvanicus), and montane voles (M. montanus). All behavioral measures were taken between January 1991 and November 1991.

Procedure

Animals were selected randomly, via a random number table, from litters, providing that at least two of the animals were of each sex. This criterion served to ensure that all subjects had previously been exposed to siblings of both sexes prior to testing. All subjects were weaned and individually caged at three weeks of age (21-22 days of age). No more than one male and female from the same litter were used in the experiment. All subjects were housed and maintained in small individual cages as previously

described. Cages were cleaned weekly by animal caretaking staff.

Odor preference tests. The olfactory preferences of each subject were tested in a repeated-measures design when they were 4, 7, and 10 weeks of age. Each test session consisted of recording selected behavioral patterns of individuals when they were presented with a simultaneous preference (choice) task that consisted of pooled bedding from unfamiliar adult males and adult females. Stimulus bedding material was pooled separately for both sexes that came from the cages of five adult males and five females that had not had their bedding changed for one week. Soiled bedding was collected in 200 cc samples from each cage and was pooled and mixed thoroughly. Small samples from the pooled bedding of each sex (approximately 20 cc) were placed in two jars (4.5 cm in diameter and 8.5 cm length) that were covered with a fine-mesh, concave screen that enabled subjects to inspect the stimulus bedding. The two jars were inserted into both ends of a large 48 X 27 X 13 cm clear plastic cage that was modified to hold the jars securely. The length of the cage was marked into three equal areas. One side contained the jar of the male bedding, the other side contained the jar with the female bedding. The third (center) area was free of any soiled bedding. The left and right placements of the male and female stimuli were randomized throughout the experiment. The apparatus had been used previously and thus validated to detect estrous

and diestrous preferences in male prairie voles (Taylor and Dewsbury, 1988), and to reveal olfactory preferences among adult montane voles (Sawrey, 1989/1990).

Each olfactory test was 10 min in duration, with the onset of the 10-min behavioral period being initiated when the subjects were observed to place their nares within 1 cm of one of the stimulus jars. Behavioral measures were recorded on a portable computer that was transported into the colony room of each species prior to testing. Subjects were tested within the dark portion of the photoperiod (1200 h - 2000 h) under the illumination of two 25-watt red light bulbs. Behavioral measures included the total duration the subject's nares were within 1 cm of each stimulus jar and total duration subjects occupied the center portion of the test cage.

Body weight and reproductive status. Body weights were recorded for subjects at the end of each behavioral test. In addition, a vaginal smear was taken from female subjects that were perforate after each behavioral test. Vaginal smears were scored under blind conditions by the author to assess the percentages of cells in each smear.

Statistical Analysis

Results of the odor preference tests were analyzed independently for each species and sex by means of two-way analysis of variances (ANOVA) with the bedding type (male or female) and week of testing comprising repeated-measure factors. Student Neuman-Keuls tests were conducted for

comparisons of means where the F value for the main effect or interaction was statistically significant. Alpha was held at .05 in all comparisons, and all were based on a two-tailed distribution. Comparisons of cell types of the vaginal smears were compared using nonparametric analyses after the cell frequencies were converted to percentages of the total cell number in each smear.

Results

Preference Tests

Few significant differences were found among the within-species comparisons of preferences for male or female bedding. Only female prairie voles and female meadow voles displayed a preference for male versus female bedding, although male pine voles, male meadow voles, female meadow voles, and male montane voles showed significant differences in the amount of time they remained near the stimuli (see below). Means and analyses for these within-species comparisons are summarized in Table 4-1 for males and Table 4-2 for females of all species.

Prairie voles: females. Female prairie voles displayed a significant preference for the male bedding compared to the female bedding, but only when viewed across the three test sessions, (main effect of stimulus, $F(1, 15) = 8.59$, $p = .010$). Post-hoc comparisons for each week did not reveal any significant preferences for the male bedding. Across the three test sessions, females remained within 1 cm of the male stimulus for 50.34 ± 11.19 s, on average, while

remaining near the female stimulus for 33.60 ± 7.02 s. They did not differ significantly in the amount of time they remained near the male and female bedding across the study, (main effect of week, $F(2, 30) = 0.59$, $p = .560$). The interaction of bedding type and week of test was not statistically significant, $F(2, 30) = 0.15$, $p = .860$ (see complete means in Table 4-2).

Meadow voles: females. Female meadow voles displayed a significant preference for the male bedding versus the female bedding, when viewed across the three test sessions (main effect of stimulus, $F(1, 17) = 12.45$, $p = .002$)(see complete means in Table 4-2). Across the three test sessions, female meadow voles remained preferentially within 1 cm of the male stimulus for 62.80 ± 10.78 s, on average, while near the female stimulus for 33.05 ± 7.47 s. Post-hoc comparisons revealed that females remained significantly longer near the male bedding than near the female bedding during week 7 (M's = 79.15 ± 13.13 s versus 37.45 ± 9.57 s). The females remained near both stimuli (collectively) significantly more during week 7 (M = 58.30 ± 11.35 s), than during week 10 (week 10, M = 38.05 ± 7.08 s)(main effect of week $F(2, 34) = 3.73$, $p = .034$). The mean duration that females were near the stimuli during week 4 was intermediate in value to those recorded during the other weeks (M = 47.42 ± 8.96 s). The interaction of bedding type and week of test was not statistically significant, $F(2, 34) = 0.84$, $p = .438$.

Differences in the Time Near the Stimuli Across Weeks

Pine voles: males. Male pine voles did not display a significant preference for either type of bedding (main effect of stimulus, $F(1, 14) = 0.009$, $p = .923$)(see means in Table 4-1). However, they remained near to the stimuli, collectively, for a longer total duration during week 4 (M = 73.40 $\pm$ 11.24 s) than during week 10 (M = 40.54 $\pm$ 12.57 s)(main effect of week, $F(2,28) = 5.98$, $p = .006$). The total duration they remained near the stimuli during week 7 was intermediate in duration to those of the other two weeks (M = 59.37 $\pm$ 12.57 s). The interaction of bedding type and week of test was not statistically significant, $F(2, 28) = 1.55$, $p = .227$.

Meadow voles: males. Male meadow voles did not display a significant preference for either type of bedding (main effect of stimulus, $F(1, 17) = 0.98$, $p = .334$)(see complete means in Table 4-1). However, their total time near the stimuli decreased significantly each week (week 4: M = 62.98 $\pm$ 8.07 s; week 7: M = 44.40 $\pm$ 9.06 s; and week 10: M = 26.08 $\pm$ 7.25 s)(main effect of week, $F(2, 34) = 17.25$, $p < .001$). The interaction of bedding type and week of test was not statistically significant, $F(2, 34) = 2.40$, $p = .105$.

Montane voles: males. Male montane voles did not display a significant preference for either type of bedding, (main effect of stimulus, $F(1, 15) = 0.64$, $p = .434$ (see complete means in Table 4-1). However, they remained near the stimuli significantly longer during week 4 (M = 113.67 $\pm$

17.69 s), than during week 7 (M = 73.63 $\pm$ 16.11 s) or week 10 (M = 72.93 $\pm$ 18.42 s) producing a main effect of week, $F(2, 30) = 7.93$, $p = .001$. The interaction of bedding type and week of test was not statistically significant, $F(2, 30) = 0.92$, $p = .40$.

Between-Species Comparisons

Between-species comparisons were conducted with two types of analysis. First, the preference ratios of male to female bedding were compared across each species independently for each sex and for each week of study (Table 4-1 and 4-2). Second, the total durations that the nares of the subjects remained within 1 cm of each stimulus were compared across each species and independently for each sex and week of study (Table 4-1 and 4-2). Nonparametric analyses (Kruskal-Wallis ANOVA by ranks tests and subsequent Mann-Whitney U tests) were used in both analyses because of heterogeneity of variances between species (see results below).

Preference ratios of males for male versus female bedding. No significant differences were found when the preference ratios of male to female bedding were compared among the males of all species for each week: week 4, $H(3, N = 64) = 2.08$, $p = .554$; week 7, $H(3, N = 64) = 2.29$, $p = .513$; week 10, $H(3, N = 64) = 1.78$, $p = .617$ (see Table 4-1 for means).

Preference ratios of females for male versus female bedding. No significant differences were found when the

ratios of male to female preferences were compared among the females of all species for each week: week 4, $H(3, N = 67) = 0.38$, $p = .944$; week 7, $H(3, N = 67) = 2.17$, $p = .536$; week 10, $H(3, N = 67) = 2.88$, $p = .410$ (see Table 4-2 for means).

Comparisons of the total durations males remained within 1 cm of the stimuli. The males of all species differed in the total duration their nares were within 1 cm of the male stimulus during each of the three tests. However, the males differed significantly in the total duration they remained near the female stimulus only during week 10 (see Table 4-1 for means).

During week 4, the male montane voles remained near the male stimulus significantly longer than the pine voles ($U = 45.0$, $p = .003$), prairie voles ($U = 56.0$, $p = .011$), and meadow voles ($U = 56.0$, $p = .002$), $H(3, N = 64) = 12.44$, $p = .006$. None of the species differed in the total time they remained near the female stimulus on week 4, $H(3, N = 64) = 4.04$, $p = .256$.

During week 7, male prairie voles remained near the male stimulus significantly more than either the pine voles ($U = 62.0$, $p = .036$) or the meadow voles ($U = 62.0$, $p = .008$), $H(3, N = 64) = 8.59$, $p = .035$. None of the species differed in total time they remained near the female stimulus, $H(3, N = 64) = 1.46$, $p = .690$.

During week 10, male meadow voles remained near the male stimulus significantly less than the prairie voles ($U = 72.0$, $p = .022$) and the montane voles ($U = 57.0$,

p = .002), H(3, N = 64) = 11.32, p = .010. Male meadow voles also remained near the female stimulus significantly less than the pine voles (U = 71.0, p = .020), prairie voles (U = 56.0, p = .004), and montane voles (U = 76.0, p = .018), H(3, N = 64) = 10.71, p = .013.

Comparisons of the total duration females remained within 1 cm of the stimuli. The females of the species revealed fewer species differences in the total duration they remained within 1 cm of the stimuli than did the males. Females were found to differ significantly in their total durations near the male stimulus during weeks 4 and 7. No species differences were found in the total durations females remained near the female stimulus during any of the tests (see Table 4-2).

During week 4, the female montane voles remained near the male stimulus significantly longer than the pine voles (U = 79.0, p = .042), prairie voles (U = 65.0, p = .006), and meadow voles (U = 90.0, p = .022), H(3, N = 67) = 9.28. None of the species differed significantly in their total time near the female stimulus, H(3, N = 67) = 7.68, p = .053.

During week 7, the female montane voles remained near the male stimulus significantly longer than the pine voles (U = 43.0, p < .001) and prairie voles (U = 65.0, p = .006), H(3, N = 67) = 15.32, p = .001. Additionally, female meadow voles remained near the male stimulus longer than did the pine voles (U = 72.0, p = .022). None of the species

differed in their total time near the female stimulus, $H(3, N = 67) = 2.15$, $p = .540$.

During week 10, none of the females of the species differed in the duration they were near the male stimulus, $H(3, N = 67) = 6.03$, $p = .110$, or the female stimulus, $H(3, N = 67) = 4.16$, $p = .244$.

Duration within Center of Cage: Within-Species Analyses

Within-species analyses of the total duration that subjects remained within the center (neutral) of the test cage revealed little systematic variation (see Table 4-3 for means and analyses of males and Table 4-4 for females). Comparisons revealed that male meadow voles remained in the center of the cage for a longer duration during weeks 4 and 7 than on week 10 (see below). The remaining sex and species differences were not significantly affected by age for this measure.

Meadow voles. Male meadow voles remained in the center of the test cage significantly longer during week 4 ($M = 94.88 \pm 11.92$) and week 7 ($M = 88.07 \pm 9.73$) than during week 10 ($M = 57.50 \pm 8.29$), $F(2, 34) = 7.03$, $p = .002$.

Duration within Center of Cage: Between-Species Analyses

Between-species comparisons revealed significant differences among the total duration males remained in the center of the test cage, although they were apparent only during week 10 (see Table 4-3 for means).

During week 10, male meadow voles remained in the center region significantly less than did the pine voles (U = 56.0, p = .004) and prairie voles (U = 59.0, p = .006), $H(3, N = 64) = 11.09$, p = .011. No differences were found among the durations males were in the center of the cage during week 4, $H(3, N = 64) = 5.46$, p = .140, or week 7, $H(3, N = 64) = 1.06$, p = .785.

There was more variation among females of the species to remain in the center of the cage than were found among the males (see means in Table 4-4). Species differences among females were found during each of the three test sessions. During week 4, female meadow voles remained in the center of the cage significantly less than the female pine voles (U = 66.0, p = .012) and prairie voles (U = 80.0, p = .027), $H(3, N = 67) = 7.85$, p = .049.

During week 7, female meadow voles remained in the center of the cage significantly less than did the female pine voles (U = 53.0, p = .003) and montane voles (U = 99.0, p = .046), $H(3, N = 67) = 10.18$, p = .017. During week 10, meadow voles remained in the center of the cage significantly less than did the pine voles (U = 41.0, $p < .001$), $H(3, N = 67) = 10.63$, p = .013.

Body Weights and Vaginal Smears

Because the analyses of body weight and vaginal cytology were secondary analyses and not the primary focus of the present study, the results of these analyses are located in Appendix D and Appendix E.

Discussion

Olfactory Preference Tests

Surprisingly few significant differences were found for olfactory preferences for male or female odors within each species at any of the weeks of testing. Female prairie voles and meadow voles revealed significant preferences to be near the male bedding when their data were pooled across the test sessions for each species. Female meadow voles revealed the highest level of attraction for the male bedding during the second test (week 7). Whether the high level of attraction by meadow voles for male bedding on week 7 is associated with a general peak of attractivity to male stimuli during the maturation period is not clear. In partial support of this possibility, female montane voles also showed a peak in attraction to the male stimulus during week 7. Thus, because the developmental rates of these two species appear to be similar (McGuire & Novak, 1984, 1986; Nadeau, 1985), the data suggest that some form of peak of male attraction occurs somewhere near week 7 of age for meadow voles and montane voles. Additional study is needed to support or refute this hypothesis.

In contrast, there was little variation in the durations female prairie voles investigated each stimulus across each week. Thus, there was no indication that prairie voles showed a gradual increase in the amount of time they remained near the male stimulus as they matured. Such a developmental pattern had been shown by female house

mice as they matured (Coppola & O'Connell, 1988; Drickamer, 1989b).

There is only limited comparative information available concerning olfactory preferences for male or female odors among species of Microtus. Ferkin and Seamon (1987) found that season influenced odor preferences in free-ranging adult female meadow voles. During the spring-summer breeding season, with long hours of daylight, both wild males and females preferred odors of opposite- versus same-sex conspecifics. However, during the late fall and winter period, which is typically a period of reproductive quiescence, females preferred female odors, whereas males did not show a preference. In other research, the preference of female meadow voles for male odors during the summer months was shown to be estrogen dependent; ovariectomy eliminated the preference, whereas estradiol reinstated the preference (Ferkin & Zucker, 1991). Thus, these studies suggest that as estrogen levels rise among female meadow voles, a preference for male versus female odors emerges. A preference for male odors may also develop as estrogen levels rise during the normal maturational process. Concurrent studies of changes in hormone levels and odor preferences across the early developmental period would clarify this possibility.

Although the males of each species did not display any olfactory preference, pine voles, meadow voles, and montane voles remained near the stimuli for different durations. In

all cases, longer durations of investigation were found in the earlier rather than later test sessions. These findings are counter-intuitive to the prediction that greater preferences would be shown as the males developed into adults. However, it is possible that some of the decreases in durations that the males remained near the stimuli were due to repeated exposure across the course of the study. I anticipated that such decreases would be minimal given the few tests imposed and the relatively long three-week interval between each test. However, because repeated-measures tests were used, it is not possible to rule out the possibility that repeated testing affected the general decline in the durations of investigation by these three species. A between-subjects design could provide evidence whether the gradual decline of investigation was due primarily to repeated testing, or whether the decline was due to changes associated with maturation.

Between-Species Comparisons

Preference ratios of males and females for soiled bedding. Comparisons of the ratios of male to female preferences between species revealed no significant differences among any of the species during any of the test sessions for each sex. However, such analyses eliminate differences in the actual amount of time that the species remained preferentially near one stimulus versus the other.

Comparisons of the total durations males were near the male and female stimuli. Few systematic differences are

discernable when comparing the total durations males were near the male stimulus. Male montane voles revealed high levels of attraction to the male stimulus compared to the other species during week 4, although the level dropped by nearly 50% by week 7. Previously, Sawrey (1989/1990) found that adult male montane voles preferred to be on the cage side containing male versus female bedding. Male meadow voles revealed relatively low levels of investigation on weeks 7 and 10 compared to the other species.

Male meadow voles were the only group to differ significantly in the duration they remained near the female stimulus. Male meadow voles displayed only a small interest in the female stimulus compared to the other species at week 10 (Table 4-1). However, this difference does not appear to be due solely to the nature of the stimulus, because they also displayed a gradual decrease in their interest to both stimuli across the study.

Comparisons of the total durations females were near the male and female stimuli. Systematic differences were more clear among the females (Table 4-2). Female montane voles remained near the male stimulus significantly longer than any of the other species during weeks 4 and 7, although no systematic differences were found among the durations females remained near the female stimulus. Because the uteri of montane voles were found to be significantly heavier among those exposed to male bedding in experiment 1, I had anticipated there would be a corresponding preference

to male bedding in Experiment 2. However, such a relationship was not found to be statistically significant.

In a previous study, Sawrey (1989/1990) found that adult female montane voles preferred to remain on the cage side containing male versus female soiled bedding. Although no significant preferences were found in the present study for female montane voles to remain near the male versus the female bedding, they did remain near the male cue on average longer than with the female stimulus across each week (Table 4-2). Together, the data from the present study and that of Sawrey's (1989/1990) suggest that female montane voles display preferences for male bedding under some conditions. It is possible that because male montane voles are territorial, females may be preferentially attracted to male odors if they are isolated from other animals and are in search of mates.

Duration with Center of Cage

Within-species comparisons of the total duration subjects remained in the center of the test cage revealed only one significant difference among the males of the species. Females of each species were not found to differ significantly on this measure. Male meadow voles remained in the center of the cage significantly longer during weeks 4 and 7 than week 10 (Table 4-3). Why this pattern would occur only among male meadow voles is not clear. The response pattern does not appear to be one of increased attraction to the stimuli, because there were concurrent and

significant decreases in the total duration that male meadow voles remained within 1 cm of the stimuli across each week (Table 4-1). Behavioral differences associated with any common pattern of development among the four species of Microtus also do not appear to explain the gradual decrease. None of the males of the other species revealed an indication of a gradual decrease in the duration they remained in the center of the cage with increasing age (Table 4-3).

Between-species comparisons of the duration subjects remained in the center of the cage revealed most differences among the females of the species. Male meadow voles remained in the center of the cage significantly less than did the pine voles or prairie voles on week 10 (Table 4-3). Female meadow voles remained in the center of the test cage significantly less than the pine voles and prairie voles during week 4, significantly less than the pine voles and montane voles on week 7, and significantly less than the pine voles on week 10 (Table 4-4). Thus, the pattern that emerges is that female meadow voles typically remained in the center of the cage for durations that were considerably less than those of the other species. During this time, female meadow voles showed high interest in male bedding across each week.

Conclusions (Experiment 2)

This exploratory study of olfactory preferences for adult female and male odors among young Microtus revealed

that few significant preferences were displayed by each species. Female prairie voles and female meadow voles revealed a small preference for the male versus the female bedding across the weeks of study. However, these few results do not enable one to make a clear evaluation of whether preferences toward the bedding are associated with differences in social or mating systems.

Two explanations seem most plausible to explain the general paucity of results. First, olfactory preferences and other behavioral differences that are influenced by olfaction may not be displayed by relatively young Microtus (those between 28 and 70 days of age). Second, this lack of preference could be due to relatively little experience with these stimuli. Richmond & Stehn (1976) suggested that prior experience or learning may significantly affect whether olfactory preferences will be displayed among some species of Microtus.

A few studies have shown that olfactory preferences occur among the adults of some species of Microtus. Olfactory preferences have been reported among adult male and female montane voles (ages 60-185 days)(Sawrey, 1989/1990), adult female meadow voles (ages 50-90 days)(Ferkin & Zucker, 1991), adult male meadow voles (ages 70-120 days of age)(Ferkin & Gorman, 1992), and adult prairie voles (specific ages not reported)(Taylor, 1988). Thus, it is possible that preferences might be detected among the species studied if they were tested at slightly

older ages or given more extensive exposure to the stimuli. The results of the present study do not suggest any clear indication that preferences were displayed during the last test session, although all species and sexes displayed a greater attraction to the male versus the female bedding on week 10 (see Tables 4-1 and 4-2). Additional study with individuals that ranged in age more widely could provide evidence whether and when preferences are shown by various species of Microtus.

An alternative explanation for the relatively few olfactory preferences observed among the species is that the subjects of all or some of the species may have preferred one stimulus compared to the other, but the preferences were not expressed because the apparatus failed to elicit them. Taylor and Dewsbury (1988) found that adult male prairie voles did not prefer bedding that had been soiled by females in estrus compared to bedding from diestrous females, providing the bedding was presented in jars. However, preferences were displayed by the males when females were tethered or placed within small cages. Thus, it is possible that slight modifications in the procedure might reveal preferences among some of the species at these ages.

Clearly, additional behavioral studies are needed to distinguish among the possibilities described above. The use of different methods to determine olfactory preferences may clarify whether which, if any, of these species display olfactory preferences for male versus soiled bedding at

young ages. In addition, such study may enable us to determine whether functional links exist between olfactory preferences and expressed social and mating systems.

Table 4-1. Mean Durations of Males (in s ± S.E.) within 1 cm of Bedding (Experiment 2).

Week:	Week 4		Week 7		Week 10		Analysis of Variance			
Odor:	Male	Famale	Male	Female	Male	Female	Factor	df	F	p
Pine Voles: Males (N = 15)										
M =	70.66	76.14	49.96	68.78	51.21	29.88	Odor	1, 14	0.009	.923
	(10.38)	(12.10)	(10.39)	(14.75)	(11.11)	(4.26)	Week	2, 28	5.98	.006**
							Inter-	2, 28	1.55	.227
% M:F[a] =	47.76		46.27		57.47		action			
Prairie Voles: Males (N = 15)										
M =	74.06	64.90	94.08	65.20	66.71	56.03	Odor	1, 14	4.08	.062
	(13.70)	(13.06)	(14.65)	(12.74)	(13.36)	(12.41)	Week	2, 28	1.17	.323
							Inter-	2, 28	0.46	.630
% M:F =	52.79		57.82		55.19		action			
Meadow Voles: Males (N = 18)										
M =	71.29	54.68	40.87	47.93	34.84	17.32	Odor	1, 17	0.98	.334
	(7.96)	(8.18)	(6.42)	(11.71)	(10.17)	(4.33)	Week	2, 34	17.25	<.001***
							Inter-	2, 34	2.40	.105
% M:F =	56.68		47.93		63.22		action			
Montane Voles: Males (N = 16)										
M =	134.79	92.56	70.89	76.37	75.89	69.97	Odor	1, 15	0.64	.434
	(19.49)	(15.90)	(14.56)	(17.65)	(12.86)	(23.99)	Week	2, 30	7.93	.001***
							Inter-	2, 30	0.92	.400
% M:F[a] =	59.95		52.94		62.28		action			

[a] % M:F Represents Mean percentage of time near male stimulus versus female stimulus.

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 4-2. Mean Durations of Females (in s ± S.E.) within 1 cm of Bedding (Experiment 2)

Week:	Week 4		Week 7		Week 10		Analysis of Variance			
Odor:	Male	Famale	Male	Female	Male	Female	Factor	df	F	p
Pine Voles: Females (N = 15)										
M =	70.34	57.57	36.49	79.98	57.93	45.71	Odor	1, 14	0.19	.669
	(16.76)	(16.32)	(7.03)	(23.41)	(16.27)	(14.45)	Week	2, 28	1.02	.373
							Inter-	2, 28	2.85	.074
% M:F =	53.66		51.72		51.48		action			
Prairie Voles: Females (N = 16)										
M =	53.26	40.41	49.61	29.70	48.17	30.69	Odor	1, 15	8.59	.010**
	(13.89)	(9.33)	(9.31)	(5.67)	(10.40)	(6.06)	Week	2, 30	0.59	.560
							Inter-	2, 30	0.15	.860
% M:F[a] =	54.00		60.06		60.32		action			
Meadow Voles: Females (N = 18)										
M =	57.59	37.25	79.16	37.45	51.66	24.45	Odor	1, 17	12.45	.002**
	(10.21)	(7.72)	(13.13)	(9.57)	(9.03)	(5.12)	Week	2, 34	3.73	.034*
							Inter-	2, 34	0.84	.438
% M:F =	56.70		65.21		69.07		action			
Montane Voles: Females (N = 15)										
M =	120.72	85.73	132.77	59.38	91.83	74.95	Odor	1, 17	2.77	.114
	(20.76)	(14.64)	(24.07)	(15.20)	(18.45)	(22.05)	Week	2, 34	1.52	.232
							Inter-	2, 34	1.78	.183
% M:F =	57.15		69.93		61.75		action			

[a]% M:F Represents Mean percentage of time near male stimulus versus female stimulus.

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 4-3. Mean Durations Males were (in sec ± S.E.) in Center of Cage (Experiment 2).

Week:	Week 4	Week 7	Week 10	Analysis of Variance Factor	df	F	p
Pine Voles: Males (N = 15)							
M =	116.05 (16.29)	97.48 (9.67)	101.10 (11.05)	Week	2, 28	0.82	.448
Prairie Voles: Males (N = 15)							
M =	99.58 (14.95)	102.85 (16.00)	111.65 (14.60)	Week	2, 28	0.31	.735
Meadow Voles: Males (N = 18)							
M =	94.88 (11.92)	88.07 (9.73)	57.50 (8.29)	Week	2, 34	7.03	.002**
Montane Voles: Males (N = 16)							
M =	68.30 (8.26)	90.24 (15.54)	83.72 (14.27)	Week	2, 30	1.17	.323

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 4-4. Mean Durations Females (in sec ± S.E.) were in Center of Cage (Experiment 2).

Week:	Week 4	Week 7	Week 10	Analysis of Variance Factor	df	F	p
Pine Voles: Females (N = 15)							
M =	125.85 (15.58)	147.85 (20.05)	128.09 (16.20)	Week	2, 28	0.73	.488
Prairie Voles: Females (N = 16)							
M =	144.52 (29.34)	113.28 (18.18)	105.64 (20.55)	Week	2, 30	1.25	.299
Meadow Voles: Females (N = 18)							
M =	73.69 (12.07)	72.29 (11.92)	61.79 (10.59)	Week	2, 34	0.48	.621
Montane Voles: Females (N = 18)							
M =	107.42 (12.77)	107.80 (15.40)	96.01 (16.82)	Week	2, 34	0.41	.664

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

CHAPTER 5
EFFECT OF SIRE PRESENCE OR ABSENCE ON DEVELOPMENT OF OFFSPRING (EXPERIMENT 3)

Rationale

One of the greatest differences between the sexes of mammalian species is the variation in their involvement in parental care (Clutton-Brock, 1991). Many species of monogamous mammals are comprised of breeding pairs where the adult male aids the female, either directly or indirectly, with the rearing of the offspring (Kleiman, 1977; Kleiman & Malcolm, 1981). However, the reasons why some species form monogamous mating systems, with extensive male parental care, while others do not, are still unknown (Dewsbury, 1987). Thus, a complete understanding of the formation of mating systems among mammals must incorporate an understanding of the evolution of parental behavior (Wittenberger, 1979).

One difficulty with understanding the evolution of male parental care in mammals is that males appear to reduce their reproductive potential by mating with only a single female, rather than seek out additional mates (Kleiman, 1977). Seeking out additional mates would appear to be the most advantageous for males, unless some other constraint, such as the spacing of females, limited a male's access to them (Emlen & Oring, 1977). However, under circumstances

where a male cannot monopolize a disproportionate share of mates, it may be to his advantage to remain with one female and to act paternally (Emlen & Oring, 1977).

Monogamy and parental care may be favored whenever more than a single individual is needed to rear the offspring (e.g., Kleiman, 1977). There is evidence that among certain species of primates, male parental care is associated with relatively high neonate:mother weight ratios (Kleiman, 1977). Thus, some species appear to have evolved a strategy of producing large offspring, or many offspring per litter, that a solitary female cannot normally rear alone (Kleiman, 1977). However, many researchers have simply assumed that paternal care has beneficial effects for the offspring; critical tests that the presence of adult males has such an effect are still needed (Wuensch, 1985).

Parental care in Microtus. Both male and female pine voles and prairie voles interact extensively with their offspring (Oliveras & Novak, 1986; Wilson, 1982), whereas in meadow voles and montane voles, the females usually spend substantially larger amounts of time interacting with the offspring than do males (Hartung & Dewsbury, 1979; Oliveras & Novak, 1986; Wang & Novak, 1992).

The consequences of parental care among Microtus have been studied to a limited extent. Wilson (1982) studied male and female meadow voles and prairie voles under laboratory conditions. Male prairie voles were more likely to huddle with the young, while females were absent from the

nest, than were male meadow voles. However, Wilson's results did not provide direct evidence that the father's presence contributed to the growth and survival of the offspring. Wilson (1982) suggested that the amount of body contact commonly observed among the members in a family group under captive conditions might produce major species differences in social structure while under natural conditions.

A preliminary study that addressed the question of whether, or how, a sires' presence may directly influence his offspring was conducted with prairie voles by Pierce and Dewsbury (1989). Their design included monitoring the development of the first and second litters of inexperienced breeding pairs. Adult males were run in a counterbalanced order so they were present either throughout the rearing of the first litter or second litter, but not both. The results indicated that the number of pups weaned, sex ratio, and body weights of surviving offspring at weaning were not significantly affected by male presence. However, females produced more offspring in the second litter when the sire had been present during the first litter. These results suggest that some measures may be useful for detecting the influence of male presence in Microtus, and that male presence may be especially beneficial for new breeding pairs.

In a related study, Storey and Snow (1987) found that among meadow voles, the presence of either the sire or

another adult male led to significant increases in the body weight of offspring compared to the weight of offspring reared alone by the female. However, in a recent investigation conducted in a seminatural environment, Wang & Novak (1992) found evidence that the presence of an adult meadow vole father, juvenile offspring, or both, exerted primarily a negative influence on the growth and development of litters. Litters that were reared only in the presence of the mother developed the most rapidly. Mother-reared offspring experienced the earliest fur and eye opening of offspring from either condition. In contrast, the same researchers found a generally positive influence of the adult father, additional juveniles, or both, when they were present with female prairie voles to rear litters. Their data revealed that prairie vole fathers contributed extensively to the rearing of the offspring by remaining in the natal nest and exhibiting parental care. Offspring that were reared with both parents, or with both parents and juveniles present, ate solid food earlier and moved out of the natal nest earlier than offspring reared without the father present. Thus, the results of the research by Wang & Novak (1992) showed virtually opposite patterns of pup development between meadow voles and prairie voles. Measures of offspring development were generally negative among meadow vole litters when additional family members were present, whereas measures of offspring development were

generally positive with additional family members present for the rearing of prairie vole litters.

Together the studies by Pierce and Dewsbury (1989), Storey and Snow (1987), and Wang and Novak (1992), suggest that there are positive effects upon the development of prairie vole offspring when adult sires, or other family members, are present during the rearing of litters. However, the results are equivocal whether paternal influence is positive or negative upon developing meadow vole offspring (Storey & Snow, 1987; Wang & Novak, 1992). It is possible that the differences found between the studies reviewed above are a function of procedural differences or other differences between the laboratories. Nevertheless, it is worthwhile to critically test whether male presence can influence the development of offspring among several species of Microtus that differ in social organization.

Although an adult sire may often be present for prairie voles or pine voles under field conditions, a sire or other adult male may be present for several species of Microtus at particular times of the year, such as during the late fall and winter months. It has been documented that winter breeding occasionally occurs in meadow voles (Madison, 1984) and montane voles (Jannett, 1984). Conceivably, any stimulus that has been available reliably throughout the evolution of a species' social and mating system could

function to influence general development and other social behaviors.

The following study was designed to assess the influence that an adult male (father) might have on the development of his offspring, among four species of voles (Microtus). Several measures were recorded in an attempt to determine which, if any, could detect differences in the effect of male presence or absence.

Measures and predictions. I predicted that male presence in the more social species, pine voles and prairie voles, would result in positive benefits upon their offspring's development. I predicted that, in comparison to litters reared alone by the females, male-present litters would weigh more at weaning, the day of eye-opening would be earlier, there would be a shorter inter-birth interval between the first and second litters, and there would be more offspring in second litters. In contrast to the assumptions for the more social species, I predicted there would be little or no positive influence upon the measures listed above for litters born to meadow voles or montane voles. Meadow voles and montane voles are not typically reared in the immediate presence of an adult male, and have a generally more rapid rate of growth than pine voles or prairie voles (McGuire & Novak, 1984, 1986; Nadeau, 1985).

Method

Subjects

A total of 364 litters born to the four species of voles were observed for differences in offspring development as a function of male presence. Breeding pairs consisted initially of sexually inexperienced adult males and females. A total of 49 pairs of pine voles (M. pinetorum), 38 pairs of prairie voles (M. ochrogaster), 61 pairs of meadow voles (M. pennsylvanicus), and 34 pairs of montane voles (M. montanus), were monitored during the development of their first and second litters. Data were gathered from newly established breeding pairs between September 1989 and June 1992.

Procedure

Each litter born to the breeding pairs was assigned randomly into one of two conditions. In the male-present condition (Together-Alone order), the first litter produced by a breeding pair developed in the presence of the dam and sire throughout the litter's first three weeks of development. The male was then removed from the female one day after the birth of the second litter. Removal of the male one day after parturition provided an opportunity for postpartum mating and subsequent pregnancies in both conditions. In the male-absent condition (Alone-Together order), the sire was removed one day after the birth of the first litter and was replaced on the day of weaning of the first litter to be present during the rearing of the second

litter. Thus, breeding pairs were treated in a counter-balanced design by removing or retaining the sire during the rearing of the first and second litters (see Figure 5-1 for graph of experimental design).

The number of offspring born in each litter was counted on the day of birth and the number that survived to day 21 (weaning) was recorded. The sexes of the surviving offspring were determined at weaning. Each offspring was weighed to the nearest 0.01 g on a pan-balance scale at weaning. In addition, the day that eye-opening occurred for the majority of offspring in a given litter (50%) was recorded.

Statistical Analysis

All measures of reproductive performance were analyzed with Analysis of Variance (ANOVA) techniques and Analysis of Covariance (ANCOVA) techniques where indicated. The results from each species were analyzed independently because of instances of heterogeneity of variances between species. The between-subject factor was the condition (order of male presence) and the repeated measure factor was the litter number (first and second). Post-hoc analyses were conducted with Neuman-Keuls post-hoc comparison tests. The probability values for the post-hoc tests are not presented in the text in order to streamline the section. The alpha level was held at .05 in all comparisons, and all comparisons were based on a two-tailed probability distribution.

Results

Delay to Produce Litters

The order of male presence (condition) significantly influenced the time required for pine voles to produce their second litters. The mean number of days that passed before breeding pairs of all species produced their first and second litters are found in Table 5-1 with the analyses. The delay of the birth of the first litter was determined by counting the number of days that passed from the initial date of pairing to the birth of the first litter. The delay until the birth of the second litter was determined by counting the number of days from the day of birth of the first litter until the birth of the second litter.

Pine voles. Pine vole pairs in both conditions gave birth to their first litters after approximately the same number of days following pairing (Together-Alone order: $M = 59.87 \pm 6.85$ days, $N = 24$; Alone-Together order: $M = 51.92 \pm 6.64$ days, $N = 25$). However, pairs in the Together-Alone order gave birth to their second litter significantly sooner than those in the Alone-Together order ($M = 32.29 \pm 2.01$ days after the first litter and $M = 53.88 \pm 4.98$ respectively). A significant interaction of condition and litter number was present, $F(1, 47) = 8.71$, $p < .005$).

Figure 5-2 displays the frequency distribution of the number of second litters born to breeding pairs of pine voles. Frequencies of litters are arranged by the number of

days that elapsed between the births of the first and second litters (see Figure 5-1). The frequency distribution reveals that most breeding pairs in the Together-Alone order (18 of 24 or 75%) gave birth to their second litter within 34 days after birth of the first litter. This time span represents pairs producing the second litter within 10 days of a normal gestation length of 24 days (Nadeau, 1985) and suggests that most of the Together-Alone pairs had successful fertilization within the post-partum estrus following the birth of the first litter.

In contrast to pairs of the Together-Alone condition, only 4 of 25 (16%) pairs in the Alone-Together condition gave birth to their second litters within 34 days of producing the first litter. Nearly half of these pairs (12 of 15 or 48%) gave birth to their second litter within 45-54 days after the birth of the first litter. Thus, this delay until birth of the second litter suggests that most of the breeding pairs in the Alone-Together condition aborted or skipped a pregnancy after the male was removed one day following the parturition of the first litter.

Because the distributions of the delay time for the pine voles to produce their first and second litters were not normally distributed in either condition, the data were compared with nonparametric analyses. Briefly, the results of the nonparametric analyses reflected those of the analysis of variance. No differences were found between the breeders of the conditions to produce their first litter

(Mann-Whitney U test: U = 248.00, p = .298), although pairs in the Together-Alone condition delivered their second litters significantly sooner than those in the Alone-Together order (U = 91.50, p < .001).

Additional ANOVA results indicated the main effect of litter number was statistically significant, F(1, 47) = 6.55, p = .013, although the main effect of condition was not, F(1, 47) = 1.31, p = .256.

Number of Offspring Born

The condition did not significantly affect the number of offspring born among any of the species for either litter, although prairie voles and montane voles had larger second litters compared to their first litters (see below). The mean number of offspring born to each species and the analyses are located in Table 5-2.

Prairie voles. The number of offspring born to prairie voles in their first and second litters was not significantly influenced by the condition, F(1, 36) = 0.57, p = .454 (see means Table 5-2). However, fewer offspring were born to prairie voles in the first litter (M = 3.29 ± .18, N = 38), than in the second litter (M = 3.81 ± .24, N = 38), which produced a significant main effect of litter number, F(1, 36) = 4.25, p = .046. The interaction of the condition and litter number was not significant, F(1, 36) = 0.51, p = .478.

Montane voles. The number of offspring born to montane voles was not significantly influenced by the condition,

$F(1, 32) = 1.42$, $p = .241$ (see means in Table 5-2). However, the average litter size was smaller in the first litter ($M = 3.88 \pm .26$, $N = 34$) than in the second litter ($M = 4.70 \pm .24$, $N = 34$), producing a main effect of litter number, $F(1, 32) = 5.42$, $p = .026$. The interaction of condition by litter number was not statistically significant, $F(1, 32) = 0.78$, $p = .383$.

Age 50% of Offspring Opened Eyes

The condition did not significantly affect the age when any of the species' offspring opened their eyes, whether the results were analyzed with or without adjusting for the number of offspring born to each litter (see means and analyses in Table 5-3). However, the eyes of the second litters produced by meadow voles opened significantly earlier than those of the first litter.

Meadow voles. The day that meadow voles opened their eyes was not significantly influenced by the condition, $F(1, 30) = 1.63$, $p = .688$ (see means Table 5-3). However, offspring from the second litter ($M = 8.03 \pm .11$, $N = 32$) opened their eyes significantly earlier than those in the first litter ($M = 8.41 \pm .15$, $N = 32$)(main effect of litter number, $F(1, 30) = 7.56$, $p = .009$). The interaction of condition by litter number was not statistically significant, $F(1, 30) = 0.57$, $p = .452$.

An analysis of covariance (ANCOVA), using the number born in each litter as a covariate, produced results similar to those of the primary analysis (main effect of condition,

$F(1, 29) = 0.15$, $p = .699$, litter number, $F(1, 29) = 7.82$, $p = .009$; and interaction of condition and litter number, $F(1, 29) = 0.44$, $p = .510$.

Number of Offspring Weaned

The number of offspring surviving from the day of birth until weaning was uniformly high for all species and for both litters; typically this was 80% or greater. The condition did not significantly affect the number of offspring weaned among any of the species, whether or not the results were analyzed with or without a covariate of the number of offspring born to each litter (see means and analyses in Table 5-4).

Sex Ratio of Offspring Weaned

The condition produced a significant main effect on the sex ratio among the montane voles. Significantly more males were born to pairs in the Together-Alone order; no significant interaction of condition by litter number was evident (see below). Mean sex ratios and analyses are located in Table 5-5.

Montane voles. The sex ratio of montane voles that were weaned were significantly influenced by the condition, (main effect of condition, $F(1, 26) = 4.73$, $p = .038$). Across both litters, montane vole pairs in the Together-Alone condition produced a greater proportion of males than females compared to those in the Alone-Together condition (M's = 61.77% $\pm$ 6.09% versus 47.18% $\pm$ 7.01%) although none of the pairwise comparisons among means by

each condition and litter were statistically different (see Figure 5-3 and Table 5-6 for means). Neither the main effect of litter number, $F(1, 26) = 2.07$, $p = .161$, nor the interaction of condition by litter number, $F(1, 26) = 0.309$, $p = .582$, was statistically significant.

An analysis of covariance (ANCOVA), using the number weaned in each litter as a covariate, produced results that were similar to those of the primary analysis (main effect of condition, $F(1, 25) = 4.38$, $p = .046$; main effect of litter number, $F(1, 25) = 1.20$, $p = .283$; and interaction of condition and litter number, $F(1, 25) = 0.32$, $p = .572$). None of the pairwise comparisons among individual means were statistically significant.

Body Weight of Offspring Weaned

The condition significantly influenced the mean body weight of pine vole offspring (see below). The analysis also indicated an influence of litter number. The mean body weights were significantly heavier in the second litters produced by the prairie voles and montane voles when they were corrected for the number of offspring weaned (see below). The mean individual body weights of offspring at weaning and their analyses are located in Table 5-6 for each species.

Pine voles. The mean body weights of individual pine voles that were weaned (Day 21) were significantly influenced by condition (interaction of condition and litter number, $F(1, 26) = 6.89$, $p = .014$). Post-hoc comparisons

revealed that offspring weights were similar during the rearing of the first litter in both conditions and not statistically different (Together-Alone: $\underline{M}$ = 11.85 $\pm$ .65, $\underline{N}$ = 16; Alone-Together: $\underline{M}$ = 13.05 $\pm$ .79, $\underline{N}$ = 12)(see Figure 5-4). However, offspring produced by pairs in the Together-Alone condition were significantly heavier in the second litter ($\underline{M}$ = 13.68 $\pm$ .63, $\underline{N}$ = 16) than in the first litter. Pairs in the Alone-Together condition weaned offspring during the second litter that were smaller than in the first litter ($\underline{M}$ = 12.38 $\pm$ .85, $\underline{N}$ = 12), but were not statistically different between litters.

Neither the main effect of condition, $\underline{F}(1, 26) = 0.003$, $\underline{p}$ = .956, nor the main effect of litter number, $\underline{F}(1, 26)$ = 1.48 $\underline{p}$ = .234, was statistically significant.

An analysis of covariance (ANCOVA), using the number of offspring weaned in each litter as a covariate, produced results similar to those of the primary analysis (main effect of male presence, $\underline{F}(1, 25) = 0.11$, $\underline{p}$ = .738; main effect of litter number, $\underline{F}(1, 25) = 2.95$, $\underline{p}$ = .097; and interaction of condition and litter number, $\underline{F}(1, 25) = 9.22$, $\underline{p}$ = .005. Post-hoc analyses revealed that only the second litters of pairs in the Together-alone order were significantly heavier than those produced in the first litter.

Prairie voles. Although the standard analysis of variance failed to detect a significant effect of condition, litter number, or interaction, the analysis of covariance

revealed a main effect of litter number, (see Table 5-6 for means and analyses).

An analysis of covariance (ANCOVA), using the number weaned in each litter as a covariate, produced results similar to those of the primary analysis (main effect of condition, $F(1, 29) = 0.26$, $p = .613$). However, a main effect of litter number was detected, $F(1, 29) = 6.64$, $p = .015$. The mean weight of prairie voles weaned during the second litter were significantly heavier than those in the first litter, despite the litter size increasing slightly between the first and second litters (first litter mean offspring weight: $M = 18.68 \pm .74$; second litter: $M = 19.08 \pm .63$). The interaction of condition and litter number was not statistically significant, $F(1, 29) = 0.48$, $p = .491$.

Montane voles. Although the standard analysis of variance failed to detect a significant effect of condition, litter number, or interaction, the analysis of covariance revealed a main effect of litter number, (see Table 5-6 for means and analyses). An analysis of covariance, using the number weaned in each litter as a covariate, failed to detect a significant main effect of condition, $F(1, 25) = 0.27$, $p = .273$. However, the mean weights of offspring weaned during the second litter were significantly heavier than those in the first litter, despite an increase in the litter size between the first and second litters (first litter mean offspring weight: $M = 15.38 + .62$; second

litter: M = 16.10 + .59)(main effect of litter number, $F(1, 25) = 5.19$, $p = .031$). The interaction of condition and litter number was not statistically significant, $F(1, 25) = 2.45$, $p = .129$.

Discussion

Although most of the results suggested little positive influence of male presence among the species, two results suggest an influence of male presence on offspring development among pine voles. The first indicates that male presence can have a substantial effect on the amount of time required before pine voles produce litters. The second result suggests that the average body weight of pine vole offspring is greater during the second litter if the male is present during the rearing of the first litter (see later section on body weight of individual offspring). Together these results suggest that continued male presence can have a positive influence on offspring development and survivorship and hence may influence the formation of the social and mating system in pine voles (see discussion below).

Delay to Produce Litters

Pine voles that were run in the Together-Alone condition produced their second litter, on average, 32 days after having the first litter (see Figures 5-1 and 5-2). However, if the males had been absent during the first litter (Alone-Together condition), the pairs typically produced their second litter 54 days after having the first

litter. This result is suggestive evidence that the continued presence of the adult sire is critical for the retention of a second pregnancy in pine voles, following post-partum mating, and may be critical for the first litter to be produced as well. In effect, male pine voles may have to remain in close proximity to a given female, even after mating, in order to successfully reproduce. This constraint could predispose males to pair monogamously with females and could lead to the evolution of paternal care, as has been documented in this species (McGuire & Novak, 1984; Schadler, 1990).

Studies have suggested that female pine voles are sensitive to changes in the social environment and will not retain pregnancies or will fail to rear existing offspring under disrupted conditions. It was reported that a substantial amount of time was necessary for new breeding pairs to become "acclimatized" to laboratory conditions before successful reproduction began (Kirkpatrick & Valentine, 1970). Schadler (1982) found that female pine voles that were exposed to strange males experienced abortions at most stages of pregnancy, even those that were lactating. In another study, the placement of an unfamiliar male with a female and a 4-day old litter led to a substantial reduction in the number of offspring that survived when compared to the survivorship of offspring if the female was left alone (Schadler, 1985). The loss of offspring occurred despite the lack of obvious wounding of

the offspring or fighting between the female and the unfamiliar male.

There is some evidence that prairie voles may also be relatively sensitive to changes in the environment during reproduction. Nadeau (1985) reported that among five species compared for levels of prenatal mortality (percent of ova lost), prairie voles had the highest rate whereas montane voles had the lowest. Pine voles were not compared in the analysis. The apparent reproductive sensitivity of pine voles, and perhaps prairie voles, to social and environmental disruptions can be contrasted with, what appear to be, lower sensitivities for disruption in some other species of Microtus. For example, in a design similar to that used by Schadler (1985) with pine voles, Storey and Snow (1987) placed unfamiliar male meadow voles with pregnant females and assessed the males' influence on litter development. Results indicated comparable survivorship and average weight gain in offspring reared either with an unfamiliar male (non-sire) or with a sire. Both groups produced more offspring that survived and were heavier compared to offspring reared by the females alone.

Number of Offspring Born

The condition (order of male presence) did not have a noticeable influence on the number of offspring produced in either litter among any of the species. However, prairie voles and montane voles had significantly larger second litters. This result appears to be a common one found among

several species of Microtus, at least for their first few litters. For example, litter size has been shown to increase with age and parity in montane voles (Negus & Pinter, 1965), but not in meadow voles (Keller & Krebs, 1970). There is some support that litter size increases across the first few litters in prairie voles, but then declines (Richmond & Conaway, 1969a). The results of the present study coincide with the results of these earlier studies.

Interestingly, litter size may be one of the attributes that responds most rapidly, and perhaps most often, to selection pressures (Schaffer & Tamarin, 1973). The only measure that was found to be statistically significant, in the study by Pierce and Dewsbury (1989) of male influence with prairie voles, was litter size. More offspring were produced in the second litter when the male had been present with the female for the rearing of the first litter. However, these results were not replicated in the present study with prairie voles.

Age 50% of Offspring Opened Eyes

The males' presence or absence did not significantly influence the time of eye opening among any of the species, although meadow vole offspring opened their eyes significantly earlier in the second litter than the first. This result might have occurred because there were more offspring born in the second litters, but some of them died before weaning. It is possible that the surviving offspring

were able to share resources, such as food and possibly body heat from the female, with fewer siblings, which in turn led to an earlier day of eye opening among the survivors. Previous study of prairie voles has shown that individual offspring weights are inversely dependent on litter size (Richmond & Conaway, 1969a).

The ages when most offspring opened their eyes in the present study are similar to values reported for eye-opening in earlier studies for each species (McGuire & Novak; 1984, 1986; Dewsbury, 1990).

Number of Offspring Weaned

Although male presence or absence did not have a significant effect on the number of offspring weaned for any species in the present study, other research has shown differential effects of male presence on the number of offspring weaned. McGuire et al. (1992) found that when adult male meadow voles (sires) remained with females to rear litters, fewer offspring survived than when the males were removed shortly after mating. In contrast, female prairie voles were generally successful at rearing all offspring produced in a litter, whether or not the male was present during rearing. It is not clear why differences were found between the McGuire et al. (1992) study and the present study. One possibility is that the breeding pairs in the McGuire et al. (1992) study had been exposed to each other periodically only for three days prior to the mating, gestation, and subsequent rearing of the first litter. In

the present study, breeding pairs remained in the same cage, throughout the rearing of the first two litters.

Sex Ratio of Offspring Weaned

The male's presence or absence was found to significantly affect the sex ratio in montane voles. Pairs in the Together-Alone condition had a greater percentage of male offspring than females, when the data were collapsed across both litters, whether the analyses were or were not corrected for differences in litter size (see Figure 5-3). However, there were no significant differences in the sex ratios when each litter was compared between each condition (post-hoc analyses).

Interpretation of this male-biased sex ratio is not straightforward. It is possible this difference was due to chance, because the effect was small and the interaction between the condition and litter number was not statistically significant. If male presence or absence affected the sex ratio in utero, we would expect larger differences in the sex ratio during the second litter because males of both conditions were present during the gestation of the first litter. Although the absolute differences in the sex ratios were larger in the second litter, the differences between the sex ratios was not statistically different during the second litter.

A few reports have indicated skews in the sex ratio of montane voles, although they were in the opposite direction found in the present study. Vaughan et al. (1973) reported

there were more female than male montane voles born within a laboratory colony. A similar finding was reported by Jannett (1981), where there were more older females than males in five wild populations of montane voles (see Nadeau, 1985). In any case, most species of Microtus appear to have litters that approximate a 1:1 sex-ratio (Nadeau, 1985). Additional study appears necessary to determine if more males than females typically are produced using these or similar procedures.

Body Weight of Offspring Weaned

Male presence appeared to substantially affect the individual offspring weights of pine voles. Mean weights were greater in the second litter than they were in first litter, when the offspring had been reared by pairs in the Together-Alone condition (see Figure 5-4). This trend remained after correcting for the number of offspring weaned in each litter. In contrast, the mean offspring weights of pine voles decreased between the litters produced by pairs in the Alone-Together condition. No significant differences in offspring weight were found between the conditions, when they were compared separately within the first and second litters.

Two interpretations seem possible for the interaction of condition and litter number or mean body weights among pine voles. First, male pine voles may have little impact during the rearing of the first litter, whether the male is or is not present. However, if the male is present during

the second litter, when he had not been present for the first, he somehow disrupts the female's ability to rear large offspring. A second interpretation is that males have a positive impact when they are present during the rearing of the first litter, but it is not revealed clearly until the second litter is produced and the male is absent.

Unfortunately, it is not possible to clearly differentiate these possibilities with the present experimental design. However, with the use of two other groups, these different hypotheses could be tested. Two additional groups, a group in which the male remains for both the first and second litters (Together-Together condition), and one in which he is present only for mating (Alone-Alone condition), could determine among the possibilities. Results for the first litter should not change; in other words, the mean weight of all groups should be similar and not statistically different during the rearing of the first litter. However, during the rearing of the second litter, the hypotheses could be tested. If offspring weights of those in the Together-Together condition were equal to or greater than offspring weights during the second litter of those run in the Together-Alone condition, this would suggest a positive influence of the male. If the offspring weights were significantly less than those in the Together-Alone condition, then it would suggest a disruptive effect of male presence.

A Reanalysis of the Evolution and Expression of Male Parental Behavior

The results of Experiment 3 suggest that some measures, recorded under certain conditions, can reveal positive or negative effects of a male's presence or absence during the rearing of pine vole litters. However, the results of many studies with other rodent species have shown that the effects are not always reliably detected (Dewsbury, 1985). A number of factors that could contribute to this problem, and possible solutions, are listed below.

First, in studies where the male is removed to determine the effect of his absence, no effect may be found because the remaining female may be capable of adjusting her behavior to compensate for the loss of the other parent (Wuensch, 1985). However, the results of the present study, and of Dewsbury's (1988), suggest that the use of repeated-measures tests, across two or more litters, might be useful to detect the gradual effect of either the loss or gain of a male's energy to the rearing of offspring. Ideally, studies would measure lifetime reproductive success of females with and without male presence, but studies of shorter duration might be sufficient to demonstrate the loss or gain of the male's contributions. For example, monitoring the development of two or three litters could be sufficient to demonstrate positive or negative effects of a male's presence or absence. Similarly, if a female must devote considerable effort to rear offspring without a male,

measures such as the time invested in eating or total calories consumed should reflect the larger energy expenditure by the female. In contrast, calories saved by a male's presence may reflect less calories consumed by females.

A second reason why the effects of male presence may not be detected readily is that males may normally contribute to the welfare of the offspring, but it is simply not observed under typical conditions of the laboratory environment (Dewsbury, 1985). For example, there is evidence that male defense of a litter may be more common than thought among some species of Microtus. Shrews (Sorex and Blarina) appear to be common predators of Microtus offspring (Pearson, 1985), but might be deterred under some circumstances. Getz et al. (1992) revealed that both sexes of prairie voles displayed aggressive behavior toward and successfully defended nestlings from short-tailed shrews (Blarina brevicauda). In contrast, female meadow voles did not behave aggressively toward the shrews or otherwise protect the nestlings. Indirect evidence suggests that male pine voles may also successfully defend their offspring against some forms of predators, such as shrews. Results of paired encounters between pine voles and meadow voles revealed that male pine voles were more aggressive than male meadow voles, and in some cases were more dominant (Cranford & Derting, 1983; Novak & Getz, 1969).

Together the results of the present study and of others suggest that for pine voles, the continued presence of a male may make the difference between the retention of a gestating litter and the survival of his current offspring. Within the present experiment, male presence appeared to be most critical for successful reproduction in pine voles, but given the undoubtedly more difficult conditions in the field, the retention of a male may be critical for successful reproduction in other species of Microtus as well. For example, relatively little is known about the social dynamics that occur during winter breeding, but it has been reported to occur among several species of Microtus, including meadow voles, prairie voles, California voles (M. californicus), and Townsend's voles (M. townsendii)(see Jannett, 1984). It may be that during harsh times as these, males contribute substantially to the success of their offspring.

The emerging view is that male paternal behavior may be expressed by many, if not all species of Microtus, at least under particular conditions (see Hartung & Dewsbury, 1979). Dewsbury (1985) noted that although stable species differences exist in the levels of paternal behavior expressed among rodents, the behavioral patterns appear to be similar in form but are expressed under varying thresholds and conditions. The next critical step appears to be to identify what conditions elicit paternal care, determine what the thresholds are, and how the benefits and

costs of paternal behavior are traded among individuals forming the social groups where it is expressed. It seems plausible that once this information is known, we may be in a better position to understand the selective pressures shaping and maintaining given social and mating strategies among Microtus.

Table 5-1. Mean Number of Days (± S.E.) until Birth of Litters (Experiment 3).

Condition:	Together-Alone		Alone-Together		Analysis of Variance			
Litter:	First	Second	First	Second	Factor	df	F	p
Pine Voles:								
M =	59.87	32.29	51.92	53.88	Condition	1, 47	1.31	.256
	(6.85)	(2.01)	(6.63)	(4.98)	Litter	1, 47	6.55	.013
N =	24	24	25	25	Interaction	1, 47	8.71	.004**
Prairie Voles:								
M =	32.11	25.06	29.50	33.60	Condition	1, 36	0.45	.502
	(7.35)	(1.58)	(2.95)	(2.51)	Litter	1, 36	0.15	.700
N =	18	18	20	20	Interaction	1, 36	2.13	.152
Meadow Voles:								
M =	27.93	28.30	31.90	27.68	Condition	1, 59	0.45	.501
	(2.24)	(2.35)	(3.43)	(1.86)	Litter	1, 59	0.54	.463
N =	30	30	31	31	Interaction	1, 59	0.77	.383
Montane Voles:								
M =	25.31	25.50	27.56	31.33	Condition	1, 32	2.42	.129
	(1.41)	(2.14)	(2.70)	(2.73)	Litter	1, 32	0.87	.356
N =	16	16	18	18	Interaction	1, 32	0.71	.403

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 5-2. Mean Number of Offspring (± S.E.) Born in Litters (Experiment 3).

Condition:	Together-Alone		Alone-Together		Analysis of Variance			
Litter:	First	Second	First	Second	Factor	df	F	p
Pine Voles:								
M =	1.62	2.00	1.60	1.56	Condition	1, 47	3.36	.072
	(.12)	(.13)	(.11)	(.10)	Litter	1, 47	2.42	.125
N =	24	24	25	25	Interaction	1, 47	3.72	.059
Prairie Voles:								
M =	3.06	3.78	3.50	3.85	Condition	1, 36	0.57	.454
	(.27)	(.36)	(.23)	(.33)	Litter	1, 36	4.25	.046*
N =	18	18	20	20	Interaction	1, 36	0.51	.478
Meadow Voles:								
M =	3.87	4.67	4.03	3.62	Condition	1, 60	1.55	.217
	(.34)	(.41)	(.29)	(.30)	Litter	1, 60	0.37	.542
N =	30	30	32	32	Interaction	1, 60	3.51	.065
Montane Voles:								
M =	3.81	4.31	3.94	5.06	Condition	1, 32	1.42	.241
	(.28)	(.39)	(.44)	(.33)	Litter	1, 32	5.42	.026*
N =	16	16	18	18	Interaction	1, 32	0.78	.383

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 5-3. Mean Age (in Days ± S.E.) when 50% or More of Offspring Opened Eyes (Experiment 3).

Condition:	Together-Alone		Alone-Together		Analysis of Variance				Analysis of Covariance			
Litter:	First	Second	First	Second	Factor	df	F	p	df	F	p	
Pine Voles:									(Covariate: Number of Offspring Born)			
M =	11.06	10.65	11.37	11.31	Condition	1, 31	2.17	.149	1, 30	2.15	.152	
	(.30)	(.23)	(.31)	(.31)	Litter	1, 31	0.97	.331	1, 30	1.09	.304	
N =	17	17	16	16	Interaction	1, 31	0.52	.472	1, 30	0.62	.434	
Prairie Voles:												
M =	8.37	8.81	8.89	8.84	Condition	1, 33	1.65	.206	1, 32	0.82	.370	
	(.24)	(.21)	(.10)	(.14)	Litter	1, 33	2.51	.122	1, 32	1.42	.241	
N =	16	16	19	19	Interaction	1, 33	4.07	.051	1, 32	3.63	.065	
Meadow Voles:												
M =	8.40	8.13	8.41	7.94	Condition	1, 30	1.63	.688	1, 29	0.15	.699	
	(.23)	(.13)	(.19)	(.16)	Litter	1, 30	7.56	.009**	1, 29	7.82	.009**	
N =	15	15	17	17	Interaction	1, 30	0.57	.452	1, 29	0.44	.510	
Montane Voles:												
M =	10.77	10.77	10.50	10.79	Condition	1, 25	0.22	.640	1, 24	0.29	.592	
	(.20)	(.23)	(.23)	(.19)	Litter	1, 25	1.06	.310	1, 24	0.003	.954	
N =	13	13	14	14	Interaction	1, 25	1.06	.310	1, 24	0.98	.331	

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 5-4. Mean Number of Offspring (± S.E.) Weaned in Litters (Experiment 3).

Condition:	Together-Alone		Alone-Together		Analysis of Variance				Analysis of Covariance			
Litter:	First	Second	First	Second	Factor	df	F	p	df	F	p	
Pine Voles:									(Covariate: Number of Offspring Born)			
M =	1.37	1.71	1.35	1.26	Condition	1, 45	2.51	.119	1, 44	0.27	.603	
	(.14)	(.18)	(.16)	(.14)	Litter	1, 45	0.52	.472	1, 44	0.008	.925	
N =	24	24	23	23	Interaction	1, 45	1.52	.223	1, 44	0.27	.603	
Prairie Voles:												
M =	2.83	3.28	3.23	3.65	Condition	1, 33	0.94	.337	1, 32	1.01	.321	
	(.34)	(.40)	(.29)	(.34)	Litter	1, 33	2.19	.148	1, 32	1.06	.310	
N =	18	18	17	17	Interaction	1, 33	0.003	.955	1, 32	1.32	.258	
Meadow Voles:												
M =	3.24	3.59	3.48	3.11	Condition	1, 54	0.09	.759	1, 53	0.37	.541	
	(.30)	(.44)	(.33)	(.37)	Litter	1, 54	0.001	.970	1, 53	1.14	.290	
N =	29	29	27	27	Interaction	1, 54	1.07	.305	1, 53	0.16	.688	
Montane Voles:												
M =	3.20	4.27	3.94	4.47	Condition	1, 30	0.98	.327	1, 29	0.05	.821	
	(.40)	(.38)	(.42)	(.51)	Litter	1, 30	3.87	.058	1, 29	0.21	.645	
N =	15	15	17	17	Interaction	1, 30	0.43	.512	1, 29	2.09	.158	

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 5-5. Mean Sex Ratio of Offspring (± S.E.) Weaned in Litters (Experiment 3).

Condition:	Together-Alone		Alone-Together		Analysis of Variance				Analysis of Covariance			
Litter:	First	Second	First	Second	Factor	df	F	p	df	F	p	
Pine Voles:									(Covariate: Number of Offspring Weaned)			
M =	66.66	50.00	68.18	77.27	Condition	1, 24	1.20	.282	1, 23	1.47	.237	
	(11.61)	(11.03)	(12.20)	(10.36)	Litter	1, 24	0.14	.705	1, 23	0.31	.577	
N =	15	15	11	11	Interaction	1, 24	1.69	.205	1, 23	1.96	.174	
Prairie Voles:												
M =	44.78	56.02	44.48	47.29	Condition	1, 29	0.40	.530	1, 28	0.45	.503	
	(8.75)	(6.41)	(7.42)	(8.09)	Litter	1, 29	0.73	.399	1, 28	0.14	.708	
N =	15	15	16	16	Interaction	1, 29	0.26	.611	1, 28	0.12	.730	
Meadow Voles:												
M =	56.04	52.08	58.69	58.19	Condition	1, 45	0.44	.509	1, 44	0.42	.516	
	(6.11)	(6.50)	(5.09)	(5.59)	Litter	1, 45	0.19	.660	1, 44	0.07	.782	
N =	24	24	23	23	Interaction	1, 45	0.11	.733	1, 44	0.01	.906	
Montane Voles:												
M =	55.12	68.43	44.23	50.14	Condition	1, 26	4.73	.038*	1, 25	4.38	.046*	
	(6.99)	(5.20)	(8.50)	(5.53)	Litter	1, 26	2.07	.161	1, 25	1.20	.283	
N =	14	14	14	14	Interaction	1, 26	0.30	.582	1, 25	0.32	.572	

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 5-6. Mean Individual Body Weight of Offspring (± S.E.) Weaned (Experiment 3).

Condition:	Together-Alone		Alone-Together		Analysis of Variance				Analysis of Covariance			
Litter:	First	Second	First	Second	Factor	df	F	p	df	F	p	
Pine Voles:									(Covariate: Number of Offspring Weaned)			
M =	11.85	13.68	13.05	12.38	Condition	1, 26	0.003	.956	1, 25	0.11	.738	
	(.65)	(.63)	(.79)	(.85)	Litter	1, 26	1.48	.234	1, 25	2.95	.097	
N =	16	16	12	12	Interaction	1, 26	6.89	.014*	1, 25	9.22	.005**	
Prairie Voles:												
M =	18.96	19.37	18.41	18.79	Condition	1, 30	0.43	.516	1, 29	0.26	.613	
	(.85)	(.55)	(.64)	(.71)	Litter	1, 30	0.67	.416	1, 29	6.64	.015*	
N =	16	16	16	16	Interaction	1, 30	0.001	.966	1, 29	0.48	.491	
Meadow Voles:												
M =	22.31	21.62	21.62	22.03	Condition	1, 45	0.02	.879	1, 44	0.01	.920	
	(.83)	(.57)	(.83)	(.71)	Litter	1, 45	0.08	.772	1, 44	0.005	.942	
N =	24	24	23	23	Interaction	1, 45	1.34	.252	1, 44	0.71	.400	
Montane Voles:												
M =	15.28	16.86	15.49	15.34	Condition	1, 26	0.91	.346	1, 25	1.25	.273	
	(.61)	(.67)	(.64)	(.51)	Litter	1, 26	1.79	.192	1, 25	5.19	.031*	
N =	14	14	14	14	Interaction	1, 26	2.61	.118	1, 25	2.45	.129	

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

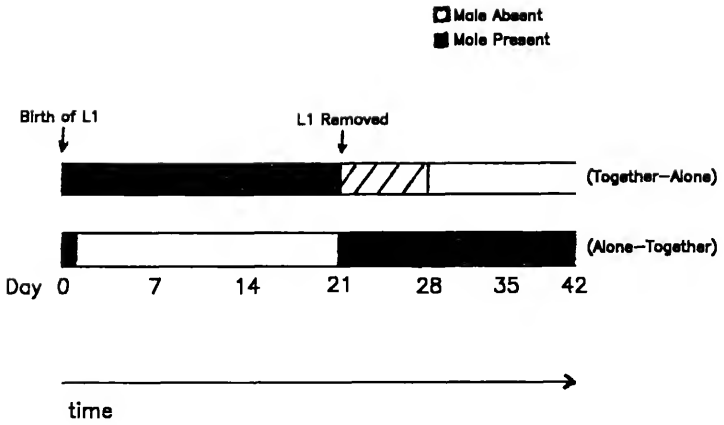

Figure 5-1. Graphical representation of the order and time of male presence and absence during Experiment 3. Together-Alone: males present during the rearing of the first litter; Alone-Together: males absent during the rearing of the first litter. L1 refers to first litter. Cross-hatched area represents variable amount of time before males were removed; males were removed one day following the birth of the second litter (see text for further explanation of conditions).

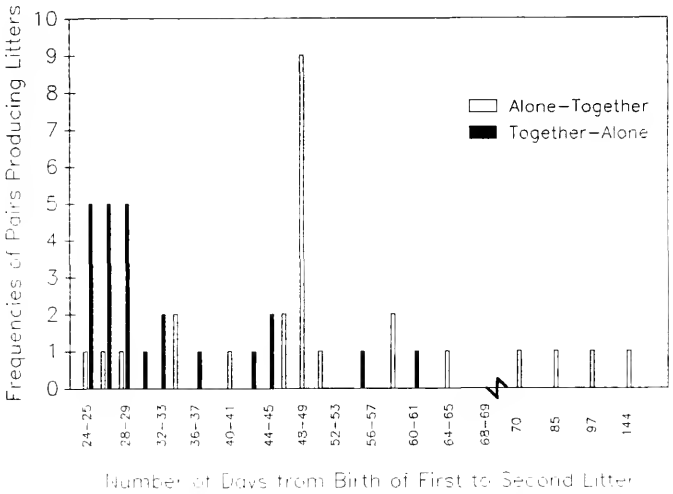

Figure 5-2. Frequency distribution of the number of breeding pairs of pine voles that produced the second litter within a given number of days from the birth of the first litter. Alone-together: males absent during the rearing of the first litter; Together-Alone: males present during the rearing of the first litter (see text for further explanation of conditions).

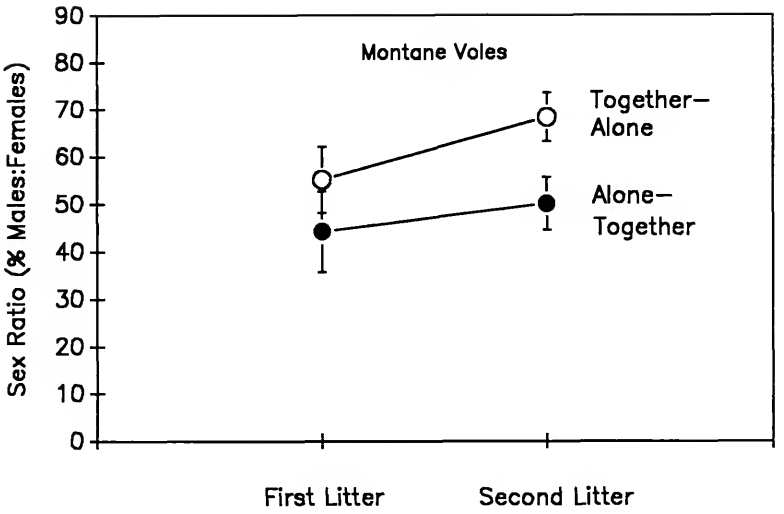

Figure 5-3. Mean sex ratio (expressed as percentages) of number of male to female offspring weaned among breeding pairs of montane voles (± standard error) as a function of the litter number. Values above 50% represent male-biased litters, those below 50% as female-biased. No significant differences were found within each litter (see text for explanation of conditions).

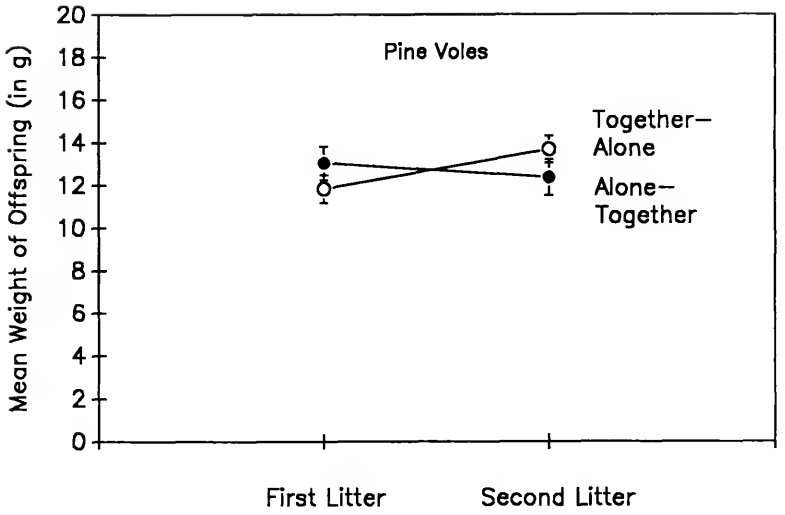

Figure 5-4. Mean individual offspring weights of pine voles at weaning (in g ± standard error) as a function of male presence and litter number (see text for explanation of conditions). Offspring produced by pairs in the Together-Alone condition weighed more in the second litter than the first. No significant differences were found between the conditions within each litter.

CHAPTER 6
GENERAL OVERVIEW AND DISCUSSION

In this final chapter I attempt to synthesize the results of the present studies, along with results from other studies, to form some possible scenarios concerning the functional and proximate means by which social and less social mating systems form among Microtus. Finally, I present an outline for routes of future study.

Overview of Experimental Results

Together the results of the present series of studies provide only limited support for the proposal that studies of puberty modulation and paternal influence may help to elucidate the evolution and maintenance of social and mating systems among species of Microtus. However, I suggest that the results of previous studies, with various species of Microtus, suggest the need for additional investigation into the relationships among puberty modulation and the formation of social and mating systems.

An Attempt at a Synthesis

No grand unifying theory is yet available that integrates the effects of puberty modulation and paternal behavior with differences in mating system among Microtus. Wittenberger (1979) suggested that no simple hypothesis or theory could be expected to explain the diversity of animal mating systems, although he believed a reasonably

comprehensive body of theory could be devised from an integration of relatively few general principles. Below I sketch out the key findings of the effects of puberty modulation and paternal behavior, from the present studies and others, that suggest relationships among these developmental processes and the formation of different social and mating systems.

Possible Relationships between Puberty Modulation, Paternal Behavior, and Social and Mating Systems

There is much evidence that shows puberty modulation occurs in house mice and several species of voles, although it has been difficult to compare meaningfully reported differences among species to determine if species differences exist and whether they vary systematically with particular ecological factors or social traits and expressed mating systems (Dewsbury, 1981). The lack of common measures and differences between laboratories have been major difficulties for this comparative analysis.

Summaries of the key social characteristics of house mice and the four species of voles that have been documented in response to social or pheromonal cues are shown in Tables 6-1 (females) and 6-2 (males). Although the methods and results of each of the studies often differ in some respects, at least two trends seem apparent. First, within the genus Microtus, evidence of puberty delay among females, and possibly males, is associated with a high degree of paternal care and a monogamous mating system. Puberty delay

being defined as the delay of some marker of puberty, or smaller weight of reproductive organs, compared to those in a control condition. A second trend is that puberty acceleration appears common among the females of all species of Microtus, regardless of the pattern of parental care and mating system of the species (puberty acceleration has been treated here as opposite to those differences listed above for puberty delay). Below I clarify each of these relationships and discuss possible ramifications of them.

Puberty delay associated with paternal care and a monogamous mating system. The relationship among sexual suppression, parental care, and a monogamous mating system is not a novel finding. Kleiman (1977) described these relationships among several mammalian species that formed a monogamous mating system. Why the relationship exists for at least two species of Microtus, pine voles and prairie voles, appears to be the most difficult question to answer. There may be several reasons which predispose various species to express these characteristics.

One of the preconditions that may favor the evolution of these traits is when group living leads to the acquisition of limited resources necessary for survival, reproduction, or both (Kleiman, 1977). The large expansive habitats of the great plains of central North America, without the pressure for long dispersal, may have selected for increased abilities to compete with others (Christian 1970). Among prairie voles, both sexes are territorial,

thus historically, breeding pairs may have had greater success defending areas in pairs or in small extended family groups. If the habitat became saturated, the evolution of puberty delay may have enabled offspring to remain within the family territory until dispersal, and may have functioned as a means of incest avoidance (Carter & Getz, 1985).

There are suggestions in the literature that offspring that are sexually suppressed, remain within a family group, and are exposed to an adult male, accrue benefits that enable them to become more reproductively successful than others that have not had the same experiences. Wang and Novak (1992) suggested two factors that may lead to the formation of extended families of prairie voles. First, offspring that help with caretaking may cause increases in the survival and quality of the young and thereby increase their inclusive fitness (Hamilton, 1964). Second, the offspring that help rear siblings may gain valuable experience, such as parental care, that may enable them to become successful breeders. Salo and French (1989) provided evidence in Mongolian gerbils that juveniles that were exposed to younger siblings, while in an extended family group, became more successful at rearing their own offspring compared to juveniles not exposed to young siblings. Specifically, the experience seemed to be most beneficial for male gerbils that had early exposure to younger siblings, rather than females.

There is other indirect evidence that experience gained within a social family group leads to enhanced reproductive success. Jakubowski and Terkel (1982) found that when wild house mice remained in a family group, along with their parents and a subsequent litter, the males later displayed paternal behavior and not typical pup-killing behavior. Similar behaviors have been shown in rats. Rosenblatt (1967) found that male laboratory rats exposed to young for several days began to display paternal responsiveness. Thus, the results of these two studies suggest that the propensity to display paternal behavior can occur as a result of exposure to a family group with young offspring. Such exposure and behavior are likely to occur in species such as prairie voles and pine voles (Getz & Carter, 1980; Schadler, 1990; Wang & Novak, 1992).

It is possible that female mate choice may operate among species that are highly social with paternal care. Theoretically, females may increase their level of fitness by selecting a mate with parental competence (Vehrencamp & Bradbury, 1984). Although studies of mate choice for parental competence have not been conducted in voles, they have been done for assessing dominance (Shapiro & Dewsbury, 1986), familiarity (Newman & Halpin, 1982; Shapiro et al. 1986) and recency of past mating (Pierce & Dewsbury, 1991). The results of all these studies have indicated that female prairie voles preferred males that were more dominant, familiar, or recently unmated. In contrast, female montane

voles did not display any systematic preference for males that differed in these qualities. Thus, it seems plausible that female prairie voles may prefer males with good care-taking capacities.

It seems plausible that females of some species of Microtus could indirectly choose mates with good care-taking qualities. Because female prairie voles and pine voles typically require longer durations of male exposure to become sexually receptive than do montane voles and meadow voles, males may have to remain in close proximity to a single female in order to mate successfully with them (Taylor et al. in press). This pattern of the male remaining within the proximity of one or few females might predispose males to behave paternally toward their progeny. This sequence of events might lead to the formation of a monogamous mating system.

In a related issue, the retention of pregnancies also appears dependent on continued male presence for some species of voles, including prairie voles (Richmond & Stehn, 1976) and montane voles (Berger & Negus, 1982; Taylor, 1990/1991). In Experiment 3, female pairmates of male pine voles that were removed after the birth of the first litter, produced a second litter significantly later than the female pairmates of those that remained in the female's presence. Thus, indirectly, the continued presence of a male may favor the formation of a monogamous mating system and serve as a means to predispose them to help with the rearing of the

offspring. It seems plausible that the evolution of sexual suppression could occur from the continued presence of the breeding male and possibly act as a means of incest avoidance among family members (Carter & Getz, 1985).

If females chose males with good caretaking abilities, and also that remain nearby to ensure estrus and pregnancy maintenance, one can envision a process where the same behaviors are expressed by their offspring. This process, in turn, could lead to the evolution of larger scale differences we see between species. Certainly these scenarios are only speculations that need additional support, but they are plausible routes of evolution for some of the differences in social and mating patterns we see today among Microtus.

Puberty acceleration appears common to all mating systems (Microtus). Puberty acceleration appears to be a ubiquitous phenomenon among the females of all four species of Microtus, but has not been demonstrated among the males (Table 6-1 and 6-2). However, it should not be ruled out that puberty acceleration does not occur in males of some species of voles. In Experiment 1, the body weights of male pine voles receiving soiled bedding from the family group or from adult males were heavier than those receiving clean bedding or bedding from adult females. However, it is not clear if changes in body weight alone should be considered a form of sexual suppression. More precise measures of reproductive activity among male Microtus may reveal cases

of acceleration (e.g., Rissman et al. 1984). Additional investigation of puberty acceleration and suppression among male Microtus should continue.

The apparent capacity for puberty acceleration among the females of many species of Microtus may have assisted in their large distribution over much of the Old and New Worlds (Tamarin, 1985). A similar capacity among house mice may have aided in their colonization. Bronson (1979) suggested that the near-global distribution of house mice was likely to have been aided substantially by their pheromonal cueing system. Certainly, more critical research is needed, with various species of Microtus, before we are able to form a greater understanding of how pheromones, puberty modulation, and the development of paternal behavior may intertwine. The great diversity of different social and mating patterns among Microtus offers a fertile resource for continued investigation.

Routes of Future Study

Together the results of these studies suggest a number of routes for future study, although with methodological precautions; these are listed below.

(1) The study of a few additional species might provide greater insight into the relationship between puberty modulation and social expression. For example, additional information gathered for field voles (M. agrestis) would seem useful because of the large amount of research previously done on the reproductive physiology of this

species (see Sawrey & Dewsbury, 1985). Information gathered on taiga voles (M. xanthognathus) may also prove valuable for comparative study. Taiga voles are considered polygynous, but unlike other species of Microtus, only the males appear to be territorial while the females are not (Wolff, 1980).

(2) Future research should be designed to identify the specific source, effective compounds, and normal means of transfer that cause changes in reproductive maturation. For example, one of the unique differences among the four species studied is the presence of hip-glands on montane voles. There is evidence that chemical cues associated with these glands are behaviorally attractive to adult males, thus they could cause changes in reproductive physiology as well (Jannett, 1978).

(3) Care must be taken to selectively control environmental stimuli in experiments that are sometimes not controlled in studies of puberty modulation. Several reports indicate significant changes in behavioral and reproductive activity as a function of changes in the photoperiod and light intensity. For example, Geyer and Rogers (1979) found that the rate of litter production of pine voles exposed to high intensity light (75-200 lumens) was nearly twice the rate under low light intensity (0-75 lumens). Similarly, Ferkin and Zucker (1991) have shown that during the spring-summer breeding season, female meadow voles prefer odors of males over those of females. However,

in the autumn-winter season of reproductive quiescence, this preference is reversed.

It is only through the selected control of stimuli such as these that we will be able to develop a clearer understanding of how olfactory stimuli affect reproductive processes, including behavior. Much experimentation, ideally interlaced with carefully designed field observations, remains to be done to clarify the relevance of puberty modulation to the social and mating strategies of Microtus.

Table 6-1. Comparison Table of Key Characteristics Among Female Muroid Rodents.

Species	House Mice	Pine Voles	Prairie Voles	Meadow Voles	Montane Voles
Social System	Deme Territory Social[1]	Monogamy Social[2]	Monogamy Social[3]	Promiscuous Asocial[4]	Polygynous Asocial[5]
Territoriality?	Males[1]	Males,[2] Females	Males,[3] Females	Females[4]	Males,[5] Females
Extensive Paternal Care?	No[1]	Yes[7]	Yes[7]	No[7]	No[9]
Puberty Delay?	Yes[15]	Yes[17,18]	Yes[8,19]	No?[8,10]	Yes?[11,24]
Puberty Acceleration via Male Cues?	Yes[13]	Yes[18]	Yes[6]	Yes[12]	Yes[16]
Puberty Acceleration via Reproductively Active Females?	Yes[14]	No? [23] (Delay)	No?[19] (Delay)	?	?
Puberty Acceleration via Females in Estrus?	Yes[15]	?	No?[20] (Delay)	?	?

1 Bronson, (1979); 2 FitzGerald & Madison, (1983); 3 Getz & Hofmann (1986); 4 Madison (1980); 5 Jannett (1980); 6 Carter et al. (1980); 7 Oliveras & Novak, (1986); 8 Batzli et al. (1977); 9 McGuire & Novak (1986); 10 Pasley & McKinney (1973); 11 Jannett (1978); 12 Baddaloo & Clulow (1981); 13 Colby & Vandenbergh (1974); 14 Drickamer & Hoover (1979); 15 Drickamer (1982); 16 Sawrey & Dewsbury (1991); 17 Schadler (1983); 18 Lepri & Vandenbergh, J. G. (1986); 19 Getz et al. (1983); 20 Carter & Getz (1985); 21 McKinney & Desjardins (1973); 22 Vandenbergh (1971); 23 Schadler (1990); 24 This study (Experiment 1).

Table 6-2. Comparison Table of Key Characteristics Among Male Muroid Rodents.

Species	House Mice	Pine Voles	Prairie Voles	Meadow Voles	Montane Voles
Social System	Deme Territory Social[1]	Monogamy Social[2]	Monogamy Social[3]	Promiscuous Asocial[4]	Polygynous Asocial[5]
Territoriality?	Males[1]	Males,[2] Females	Males,[3] Females	Females4	Males,[5] Females
Extensive Paternal Care?	No[1]	Yes[7]	Yes[7]	No[7]	No[9]
Puberty Delay?	Yes[21,22]	Yes?[23]	Yes?[8]	No?[8]	No?[11]
Puberty Acceleration via Female Cues?	Yes[22]	No?[24]	No?[24]	No?[24]	No?[24]
Puberty Acceleration via Reproductively Active Females?	?	?	?	?	?
Puberty Acceleration via Females in Estrus?	?	?	?	?	?

1 Bronson, (1979); 2 FitzGerald & Madison, (1983); 3 Getz & Hofmann (1986); 4 Madison (1980); 5 Jannett (1980); 6 Carter et al. (1980); 7 Oliveras & Novak, (1986); 8 Batzli et al. (1977); 9 McGuire & Novak (1986); 10 Pasley & McKinney (1973); 11 Jannett (1978); 12 Baddaloo & Clulow (1981); 13 Colby & Vandenbergh (1974); 14 Drickamer & Hoover (1979); 15 Drickamer (1982); 16 Sawrey & Dewsbury (1991); 17 Schadler (1983); 18 Lepri & Vandenbergh, J. G. (1986); 19 Getz et al. (1983); 20 Carter & Getz (1985); 21 McKinney & Desjardins (1973); 22 Vandenbergh (1971); 23 Schadler (1990); 24 This study (Experiment 1).

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APPENDIX A
MEANS OF BODY WEIGHTS AND NUMBERS OF SUBJECTS *MICROTUS* (EXPERIMENT 1)

The following tables contain the mean body weights of subjects in Experiment 1. See text for accompanying explanation and discussion.

Appendix A. Mean Body Weight (in g ± S.E.) of *Microtus* (Experiment 3).

Condition	N	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
Pine Voles: Males								
Control	17	11.26 (.46)	14.82 (.49)	17.81 (.54)	19.53 (.55)	20.17 (.56)	21.24 (.53)	21.24 (.53)
Family	16	11.49 (.59)	15.56 (.69)	18.65 (.69)	20.68 (.72)	21.50 (.79)	22.34 (.81)	22.71 (.88)
Male	14	12.61 (.62)	16.62 (.67)	19.22 (.86)	20.78 (.90)	21.94 (.96)	22.51 (1.01)	22.96 (1.05)
Female	16	11.77 (.60)	15.64 (.56)	17.63 (.59)	18.95 (.59)	19.76 (.62)	20.29 (.59)	20.64 (.65)
Prairie Voles: Males								
Control	16	18.48 (.44)	24.66 (.75)	26.85 (.92)	28.37 (1.04)	29.80 (1.17)	30.69 (1.29)	31.94 (1.41)
Family	16	20.53 (.65)	25.49 (.78)	27.79 (.89)	29.34 (1.07)	30.47 (1.13)	31.44 (1.17)	32.42 (1.28)
Male	16	18.68 (.53)	24.45 (.79)	26.53 (1.03)	28.04 (1.26)	29.65 (1.34)	31.00 (1.41)	31.74 (1.46)
Female	16	18.46 (.65)	24.04 (.80)	25.72 (.97)	27.17 (1.07)	28.64 (1.17)	29.77 (1.28)	30.90 (1.35)

Appendix A--continued. Mean Body Weight (in g ± S.E.) of *Microtus* (Experiment 3).

Condition	N	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
Meadow Voles: Males								
Control	16	21.23 (.94)	26.93 (1.14)	29.43 (1.25)	32.54 (1.52)	35.22 (1.74)	37.83 (1.99)	39.18 (2.14)
Family	16	21.89 (.80)	27.26 (1.07)	29.95 (1.25)	33.42 (1.45)	36.89 (1.66)	39.67 (1.85)	41.64 (1.97)
Male	16	23.16 (.55)	28.73 (.45)	31.50 (.67)	35.45 (.89)	38.99 (.94)	41.56 (1.16)	43.49 (1.28)
Female	16	20.35 (.58)	27.07 (.72)	30.68 (1.02)	34.44 (1.32)	37.90 (1.37)	41.09 (1.50)	43.13 (1.58)
Montane Voles: Males								
Control	16	16.09 (.87)	23.27 (1.05)	27.74 (1.11)	31.65 (1.28)	34.47 (1.45)	36.54 (1.63)	37.70 (1.67)
Family	16	17.29 (.94)	25.19 (1.03)	28.80 (1.14)	31.29 (1.40)	33.61 (1.48)	35.52 (1.50)	37.22 (1.51)
Male	16	17.22 (.87)	24.03 (.88)	27.81 (1.15)	31.39 (1.46)	34.71 (1.61)	37.21 (1.83)	39.12 (2.04)
Female	16	18.04 (.80)	24.76 (.77)	28.46 (1.09)	32.21 (1.30)	34.80 (1.27)	36.65 (1.32)	38.64 (1.35)

Appendix A--continued. Mean Body Weight (in g ± S.E.) of *Microtus* (Experiment 3).

Condition	N	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
Pine Voles: Females								
Control	16	10.68 (.55)	13.81 (.48)	16.40 (.48)	17.79 (.55)	18.38 (.53)	18.66 (.54)	18.87 (.64)
Family	16	11.89 (.62)	15.47 (.55)	17.96 (.74)	19.35 (.83)	20.31 (.92)	21.13 (.99)	21.40 (1.03)
Male	18	11.85 (.61)	15.62 (.62)	17.90 (.67)	19.26 (.66)	20.04 (.69)	20.59 (.69)	20.88 (.73)
Female	16	11.50 (.77)	14.95 (.67)	17.37 (.66)	18.94 (.72)	19.83 (.76)	20.04 (.80)	20.45 (.80)
Prairie Voles: Females								
Control	18	18.12 (.63)	22.07 (.56)	23.13 (.66)	23.95 (.79)	25.01 (.82)	25.80 (.99)	26.65 (1.03)
Family	16	17.46 (.68)	21.91 (.64)	23.09 (.61)	24.14 (.65)	24.92 (.69)	25.89 (.71)	26.58 (.74)
Male	16	18.11 (.57)	22.40 (.61)	23.83 (.82)	24.89 (.98)	26.14 (1.16)	26.96 (1.31)	27.91 (1.31)
Female	16	18.12 (.61)	22.03 (.57)	23.28 (.63)	24.32 (.75)	25.00 (.80)	25.90 (.90)	26.78 (.93)

Appendix A--continued. Mean Body Weight (in g ± S.E.) of *Microtus* (Experiment 3).

Condition	N	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
Meadow Voles: Females								
Control	16	21.91 (.79)	25.26 (.82)	26.02 (.92)	27.39 (1.02)	29.06 (1.06)	29.89 (1.23)	30.95 (1.30)
Family	15	19.87 (.84)	24.34 (.90)	24.92 (1.05)	26.67 (1.21)	28.28 (1.24)	29.56 (1.28)	30.65 (1.34)
Male	16	20.75 (.66)	24.61 (.66)	25.51 (.73)	27.15 (.83)	29.17 (.90)	30.83 (1.14)	32.02 (1.14)
Female	15	20.61 (.90)	24.50 (.98)	26.31 (1.16)	28.13 (1.34)	29.99 (1.43)	31.69 (1.58)	32.79 (1.55)
Montane Voles: Females								
Control	16	16.79 (.74)	22.24 (.78)	23.84 (.81)	25.25 (1.03)	26.65 (1.19)	27.76 (1.36)	28.92 (1.43)
Family	16	16.44 (.70)	21.95 (.90)	23.78 (1.07)	25.71 (1.28)	27.61 (1.44)	29.08 (1.61)	30.16 (1.71)
Male	15	15.87 (.60)	21.38 (.71)	23.04 (.82)	24.75 (1.06)	26.13 (1.21)	27.25 (1.37)	28.37 (1.43)
Female	16	16.84 (.64)	21.99 (.65)	23.31 (.77)	24.39 (.99)	25.53 (1.23)	26.82 (1.37)	27.67 (1.44)

APPENDIX B
MEANS OF ANOGENITAL DISTANCE (IN MM) IN *MICROTUS* (EXPERIMENT 2)

The following tables contain the mean anogenital distances, standard errors of the mean, and numbers of males of the four *Microtus* species studied in Experiment 1. See text for further discussion of the results.

Appendix B. Mean Anogenital Distance (in mm ± S.E.) of Male *Microtus* (Experiment 3).

Condition	N	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
Pine Voles								
Control	17	4.32 (.29)	5.21 (.26)	6.15 (.24)	6.68 (.27)	7.21 (.20)	7.56 (.32)	7.71 (.22)
Family	15	4.47 (.28)	5.50 (.21)	6.07 (.27)	6.23 (.30)	7.00 (.25)	7.40 (.26)	7.70 (.26)
Male	14	4.75 (.17)	5.75 (.21)	6.75 (.32)	6.86 (.27)	7.00 (.26)	7.82 (.31)	7.96 (.25)
Female	16	4.62 (.22)	5.41 (.24)	5.66 (.24)	6.41 (.22)	6.94 (.28)	6.97 (.18)	7.75 (.24)
Prairie Voles								
Control	15	7.53 (.34)	9.93 (.27)	11.03 (.38)	11.37 (.41)	11.67 (.39)	11.73 (.50)	12.20 (.38)
Family	16	8.28 (.32)	10.12 (.33)	10.97 (.43)	11.91 (.39)	11.66 (.40)	11.91 (.31)	12.31 (.32)
Male	16	8.12 (.34)	10.37 (.26)	11.06 (.31)	11.53 (.33)	11.60 (.27)	11.97 (.35)	12.28 (.35)
Female	16	7.75 (.22)	9.66 (.31)	10.53 (.31)	10.94 (.41)	11.31 (.37)	11.12 (.35)	11.25 (.36)

Appendix B--continued. Mean Anogenital Distance (in mm ± S.E.) of Male *Microtus* (Experiment 3).

Condition	N	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
Meadow Voles								
Control	16	10.94 (.49)	12.81 (.44)	14.97 (.47)	16.97 (.55)	17.19 (.72)	18.09 (.62)	18.22 (.61)
Family	16	10.47 (.39)	12.94 (.44)	15.16 (.59)	15.81 (.44)	16.81 (.53)	17.69 (.56)	18.09 (.50)
Male	16	11.16 (.37)	13.09 (.41)	15.78 (.49)	16.53 (.59)	17.62 (.51)	17.91 (.61)	19.19 (.55)
Female	16	10.65 (.47)	13.09 (.47)	14.72 (.58)	17.16 (.65)	18.12 (.64)	18.16 (.59)	18.72 (.74)
Montane Voles								
Control	16	7.00 (.38)	8.81 (.52)	10.31 (.45)	12.19 (.53)	12.75 (.69)	12.69 (.37)	13.44 (.54)
Family	16	7.00 (.36)	9.59 (.45)	10.53 (.48)	11.62 (.38)	11.68 (.30)	11.72 (.29)	12.25 (.31)
Male	16	6.87 (.44)	8.47 (.47)	10.75 (.45)	10.91 (.44)	11.84 (.60)	12.19 (.48)	13.09 (.48)
Female	16	7.50 (.32)	9.41 (.45)	10.66 (.46)	11.25 (.55)	12.28 (.37)	11.97 (.37)	12.66 (.36)

APPENDIX C
ANALYSES OF NUCLEATED CELLS AND LEUKOCYTES FROM VAGINAL SMEARS OF MICROTUS (EXPERIMENT 1)

Appendices C-1 through C-3 contain the number of subjects and the mean percentages of nucleated, and leukocytes for each species in Experiment 1. These analyses of nucleated cells and leukocytes complement the results reported in the primary text for cornified cells (see Vaginal Smears of results section for methods).

Within-Species Comparisons of Cell Types

Prairie voles: nucleated cells: condition effects: Few significant differences were found among the percentages of nucleated cells from prairie voles as a function of condition. On Days 43-44, the mean percentage of cells in the Control condition (M = 3.8%) was significantly less than those in the Family and Male conditions (M's = 9.3% and 7.8% respectively), (Kruskal-Wallis ANOVA: $H(3, N = 62) = 7.85$, $p = .049$). The number of nucleated cells in the Female condition were intermediate in value to the others (M = 6.7%). A second significant effect was found for Days 51-52; the mean percentage of nucleated cells from the Male condition (M = 11.5%) was significantly higher than those in either the Control or Female conditions (M's = 6.3% and 5.0%), $H(3, N = 64) = 9.27$, $p = .026$.

Age effects. In the repeated measures analyses, the percentages of nucleated cells from prairie voles in the Female condition shifted significantly across alternate blocks of days, although not in any clear, systematic fashion (Friedman ANOVA: $X^2(7, N = 15) = 16.21, p < .023$). Levels of nucleated cell were relatively high on days 33-34 ($M = 8.5\%$), then decreased in level by Day 41-2 ($M = 4.6\%$) and then rose again to the highest level ($M = 9.6\%$) on Days 49-50. (Note: differences below are indicated by direction of later days being higher in cell percentages than earlier days except where noted "*"). Other significantly different blocks of days from those in the Female condition included: Days 33-34 versus 37-38*, Days 37-38 versus Days 49-50; Days 41-42 vs. 49-50; and Days 49-50 versus Day 57-58*.

Changes in the percentages of nucleated cells across days, among prairie voles in the other three conditions, did not reach statistically significant levels.

Leukocytes: condition effects. Relatively small changes in the percentages of leukocytes among prairie voles resulted in only one significant shift among the blocks of days. During Days 35-36, significantly more leukocytes were found in the Control and Female conditions than in the Family condition (M's = 78.2% and 83.3% versus 67.7%), $H(3, N = 55) = 9.72, p = .021$.

Age effects. Repeated measures analyses for leukocytes mirrored those of cornified cells. No significant shifts were found among any of the four experimental conditions.

Meadow voles: nucleated cells: condition effects. Analyses of the percentages of nucleated cells among the conditions did not reveal any statically significant differences.

Age effects. Only meadow voles in the Female condition displayed significant shifts in the percentages of nucleated cells, $X^2(7, N = 13) = 17.42$, $p < .015$. Typically there were decreasing proportions of nucleated cells as the females in Female condition grew older. Specific significant differences in the percentages of nucleated cells included: Days 37-38 versus 49-50* through 61-62*; Days 41-42 versus 49-50*, 57-58 through 61-62*; and Days 45-46 versus 57-58* through 61-62*.

Leukocytes: condition effects. Comparisons of the percentages of leukocytes by blocks of days among the different conditions did not reveal any statically significant differences.

Age effects. Significant changes in the percentages of leukocytes were evident as a function of age. These changes in percentages largely reflected, inversely, the differences reported in the main text for the percentages of cornified cells. Analyses revealed there were typically decreasing proportions of leukocytes, in all conditions, across the course of the study. All conditions had significant changes in the percentage of leukocytes.

Control condition. Subjects in the Control conditioned had the highest percentages of leukocytes during the first

blocks compared to latter days (Day 33-34 and 37-38; M's = 80.3% and 81.2%) ($X^2(7, N = 11) = 28.73, p < .001$). Specific differences included: Day 33-34 versus Days 49-50 through Days 61-62*; Days 37-38 versus Days 41-42 through Days 61-62*; Days 45-46 versus Days 53-54 through Days 61-62*.

Family condition. Percentages of leukocytes were higher in the earlier days of observation than later, $X^2(7, N = 11) = 32.76, p < .001$. Specific pairwise comparisons that were significantly different include: Days 33-34 versus Days 45-46 through Days 61-62*; Days 37-38 versus Days 45-46 through Days 61-62*; Days 41-42 versus Days 45-46 through Days 61-62*; Days 37-38 versus Days 41-42*.

Male condition. Percentages of leukocytes were higher in the earlier days of observation than later, $X^2(7, N = 12) = 36.11, p < .001$. Significant differences included: Days 33-34 versus Days 41-42 through Days 61-62*; Days 37-38 versus Days 49-50 through Days 61-62*; Days 41-42 versus Days 57-58 through Days 61-62*; and Days 45-46 versus Days 53-54 through Days 61-62*.

Female condition. Percentages of leukocytes were higher in the earlier days of observation than later, $X^2(7, N = 13) = 46.15, p < .001$. Significant differences included: Days 33-34 versus Days 45-46 through Days 61-62*; Days 37-38 versus Days 45-46 through Days 61-62*; Days 41-42 versus Days 45-46 through Days 61-62*; and Days 45-46 versus Days

53-54 through Days 61-62*; and Days 49-50 versus Days 57-58*.

Montane voles: nucleated cells: condition effects. Variations in the percentages of nucleated cells among the different conditions were minimal and resulted in only one significant result among the groups on Days 61-62, $H(3, N = 62) = 9.78$, $p = .020$. On this block, the mean percentage of nucleated cells was significantly greater from subjects in the Female condition ($M = 6.9\%$) compared to the percentages of nucleated cells from those in either the Control ($U = 50.0$, $p = .01$) or Family conditions ($U = 45.0$, $p = .003$)(M's = 4.6% and 4.0%).

Age effects. Repeated measures analysis revealed that only one condition resulted in significant differences in the percentages of nucleated cells. Subjects of the Family condition had significant change in the proportions of nucleated cells, $X^2(7, N = 11) = 14.21$, $p < .047$. Post-hoc comparisons indicated that all days, except Days 53-54, had significantly more nucleated cells than on Day 61-62 ($M = 3.3\%$). The cell percentages were relatively small among the earlier blocks of days, (M's range: 5.0% to 8.7%), but were significantly more than the last block of Days 61-62.

Leukocytes: condition effects: Three of the last five two-day blocks of days were found to have significant differences in the percentages of leukocytes among the conditions (Days 53-54: $H(3, N = 61) = 8.77$, $p = .032$; Days

57-58: $H(3, N = 62) = 8.47$, $p = .037$; Days 61-62: $H(3, N = 62) = 9.17$, $p = .027$).

Post-hoc comparisons of the cell percentages for Days 53-54 revealed that the Control condition had significantly more leukocytes than those in the Female condition (M's = 61.1% and 41.0%; U = 64.0, $p = .046$), those in the Family condition had significantly more cells than those in the Male condition (M's = 56.3% versus 53.0%; U = 66.0, $p = .033$), and those in the Male condition had more than those in the Female condition (M's = 53.0% versus 41.0%; U = 55.0, $p = .029$).

Analyses of Days 57-58 revealed only one significant difference among the conditions, those in the Control condition had significantly more leukocytes than those in the Family condition (M's = 60.5% versus 53.9%; U = 79.0, $p = .040$). Those in the Control and Female failed to be significantly different ($p = .051$).

On days 61-62, two statistical differences were found. Those in Control condition had a greater percentage of cells than those in the Family condition (M's = 60.5% versus 57.7%; U = 72.0, $p = .021$), and those in the Family condition had significantly more leukocytes than those in the Male condition (M's = 57.7% versus 49.7%; U = 70.0, $p = .030$). Those in the Control and Female failed to differ significantly ($p = .051$).

Leukocytes: age effects: Comparisons of the percentages of leukocytes among the different conditions revealed

significant differences within each group. Statistical differences were found among blocks of days including Days 53-54, 57-58, and 61-62. These blocks of days were the same blocks where differences were found among the percentages of cornified cells (see main text).

Control condition. Percentages of leukocytes were higher in the earlier days of observation than later, $X^2(7, N = 14) = 20.48$, $p < .004$. Specific differences included: Days 33-34 versus Days 41-42 and Days 49-50 through Days 61-62*; Days 37-38 versus Days 57-58 through Days 61-62*; Days 41-42 versus Days 57-58 through Days 61-62*; and Days 45-46 versus Days 53-54 through Days 61-62*; and Days 49-50 versus Days 61-62*.

Family condition. Percentages of leukocytes were higher in the earlier days of observation than later, $X^2(7, N = 11) = 18.12$, $p < .011$. Significantly different blocks of days included: Days 33-34 versus Days 57-58 through Days 61-62*; Days 41-42 versus Days 49-50 through Days 61-62*; and Days 53-54 versus Days 57-58*.

Male condition. Percentages of leukocytes were higher in the earlier days of observation than later, $X^2(7, N = 12) = 23.14$, $p < .002$. Significantly different blocks of days included: Days 33-34 versus Days 49-50 through Days 61-62*; Days 37-38 versus Days 53-54, Days 61-62*; Days 41-42 versus Days 53-54, Days 61-62*; and Days 49-50 versus Days 53-54, Days 61-62*.

Female condition. Percentages of leukocytes were higher in the earlier days of observation than later, $X^2(7, N = 8) = 20.96$, $p < .004$. Days 33-34 versus Days 41-42 through Days 49-50, Days 57-58 through Day 61-62*; Days 37-38 versus Days 49-50 through Days 61-62*; Days 45-46 versus Days 61-62*; and Days 53-54 versus Days 61-62*; and Days 57-58 versus Days 61-62*.

Appendix C-1. Summary Table of Mean Percentages of Nucleated Cells and Leukocytes for Prairie Voles (Experiment 1).

	Condition											
	Control			Family			Male			Female		
Days	Nuc	Leuk	N	Nuc	Leuk	N	Nuc	Leuk	N	Nuc	Leuk	N
21-22	13.1	72.0	3	10.4	78.0	2	13.2	72.4	3	21.1	64.9	3
23-24	11.4	64.5	4	11.3	74.6	4	10.1	66.1	4	11.1	62.7	5
25-26	9.6	74.0	7	11.4	67.1	8	13.8	68.0	10	13.0	65.0	8
27-28	7.0	70.3	7	13.1	66.1	8	9.8	72.3	11	10.3	62.2	9
29-30	7.0	74.6	7	8.7	73.9	9	10.4	70.9	14	7.4	72.1	12
31-32	4.9	77.8	10	6.3	80.2	10	8.1	73.0	14	5.2	77.4	13
33-34	6.8	76.2	13	6.9	77.9	10	10.2	66.6	14	8.5	77.0	15
35-36	6.8	78.2	13	10.2	67.7	12	7.2	73.8	15	4.0	83.3	15
37-38	5.3	81.2	14	7.0	77.6	13	9.0	70.2	16	4.8	82 2	16
39-40	6.9	79.4	14	8.5	75.7	13	6.2	76.2	16	6.3	79.7	16
41-42	6.1	77.7	15	7.8	77.9	15	9.0	77.0	16	4.6	82.2	16
43-44	3.8	78.9	15	9.3	71.6	15	7.8	76.7	16	6.7	77.4	16
45-46	6.4	74.5	15	7.7	78.5	15	8.8	76.8	16	7.9	78.0	16
47-48	6.1	78.6	16	6.1	79.1	15	9.8	78.4	16	6.5	80.9	16
49-50	7.6	70.8	16	8.8	76.5	15	9.7	77.2	16	9.6	77.0	16
51-52	6.3	75.4	17	7.3	77.9	15	11.5	72.3	16	5.0	82.3	16
53-54	5.9	77.3	17	9.1	76.1	15	8.3	80.1	16	7.2	81.3	16
55-56	7.2	79.5	17	7.8	75.4	15	10.9	72.4	16	6.9	82.8	16
57-58	8.6	74.7	17	11.5	74.0	15	10.0	78.8	16	7.1	82.1	16
59-60	6.7	76.0	17	8.3	75.9	15	11.0	74.4	16	7.7	78.5	16
61-62	8.7	78.1	17	10.8	77.3	15	11.1	75.2	16	6.1	79.6	16

Nuc: Nucleated Cells; Leuk: Leukocytes.

Appendix C-2. Summary Table of Mean Percentages of Nucleated Cells and Leukocytes for Meadow Voles (Experiment 1).

	Condition											
	Control			Family			Male			Female		
Days	Nuc	Leuk	N	Nuc	Leuk	N	Nuc	Leuk	N	Nuc	Leuk	N
21-22	10.1	64.9	10	7.3	45.5	7	5.2	61.3	12	8.7	56.7	9
23-24	11.7	73.4	11	7.6	77.2	10	5.7	74.8	12	7.9	83.7	12
25-26	7.7	75.2	11	7.9	77.5	10	5.2	84.1	12	6.6	83.8	13
27-28	4.4	80.5	11	6.4	73.4	10	4.7	81.1	12	4.1	84.6	13
29-30	3.3	85.0	11	4.8	73.4	11	5.4	79.4	12	4.1	85.4	13
31-32	6.0	77.7	11	3.9	74.7	11	3.5	81.8	12	4.6	83.7	13
33-34	2.6	80.3	11	4.3	75.2	11	4.0	76.1	12	4.0	82.2	13
35-36	3.2	75.9	11	4.2	75.5	11	4.9	71.4	12	7.3	76.5	13
37-38	3.0	81.9	12	4.2	73.8	12	5.5	75.1	12	6.1	75.4	13
39-40	4.5	78.4	12	4.5	70.6	12	5.4	67.8	12	7.0	69.4	13
41-42	6.6	65.4	12	2.4	79.6	12	4.7	62.8	12	5.5	66.6	14
43-44	4.0	73.7	12	3.0	73.5	12	4.3	62.6	12	4.2	62.5	14
45-46	4.6	72.0	12	6.2	59.3	12	4.5	64.2	12	4.9	57.1	14
47-48	5.7	67.8	12	5.5	59.0	12	3.3	52.4	12	3.8	52.8	14
49-50	2.5	66.8	12	5.7	55.4	13	2.5	57.8	13	2.8	50.0	14
51-52	4.3	63.7	13	4.5	56.5	13	4.0	56.0	13	4.5	47.9	14
53-54	6.7	61.1	13	5.4	56.3	13	3.6	53.0	13	2.8	41.0	14
55-56	5.9	54.2	13	3.1	61.3	13	3.5	54.5	13	4.5	43.7	14
57-58	4.4	60.5	13	4.7	53.9	13	3.0	56.3	13	2.5	42.3	14
59-60	4.4	58.8	14	4.1	54.3	13	5.0	53.8	15	3.4	39.9	14
61-62	4.2	60.5	14	4.8	57.7	13	4.3	49.7	15	2.6	43.1	14

Nuc: Nucleated Cells; Leuk: Leukocytes.

Appendix C-3. Summary Table of Mean Percentages of Nucleated Cells and Leukocytes for Montane Voles (Experiment 1).

	Condition											
	Control			Family			Male			Female		
Days	Nuc	Leuk	N	Nuc	Leuk	N	Nuc	Leuk	N	Nuc	Leuk	N
21-22	16.6	66.3	7	16.6	68.6	7	13.2	73.9	8	13.3	78.7	6
23-24	10.2	68.4	9	10.2	78.8	7	12.5	71.6	10	11.9	73.8	9
25-26	9.8	74.4	10	9.6	72.9	7	10.7	72.1	10	16.6	67.4	9
27-28	10.7	71.3	10	6.5	80.7	8	11.4	72.2	11	8.1	71.1	9
29-30	7.9	58.6	10	8.9	70.0	8	13.8	68.2	11	4.9	79.2	9
31-32	4.3	57.4	11	8.3	68.3	8	12.6	68.0	11	5.8	77.2	9
33-34	7.6	55.5	14	8.6	65.1	11	10.1	57.6	12	4.8	77.9	9
35-36	6.2	57.8	15	10.5	66.7	11	5.8	64.7	12	4.1	80.0	9
37-38	8.4	51.1	15	8.0	62.1	12	10.0	50.7	12	8.2	73.7	9
39-40	7.4	53.9	15	7.2	63.3	12	8.0	51.4	12	9.1	70.0	10
41-42	8.6	48.2	15	6.8	64.7	12	7.8	56.2	14	8.9	66.4	9
43-44	10.2	46.4	16	9.4	56.5	13	8.0	57.8	14	9.6	68.4	11
45-46	6.1	52.7	16	7.6	57.7	14	6.1	54.0	14	6.7	63.5	13
47-48	6.4	43.6	16	7.4	58.8	15	7.1	55.8	14	8.4	66.2	13
49-50	6.1	46.4	16	8.2	56.1	15	10.0	46.8	15	9.3	62.4	14
51-52	5.9	39.7	16	6.6	58.4	15	7.7	49.0	15	8.8	59.5	13
53-54	5.2	43.5	16	6.2	61.7	16	6.2	38.8	15	7.3	60.6	14
55-56	6.2	41.3	16	7.3	50.8	16	5.4	51.1	15	7.6	60.6	14
57-58	5.9	38.0	16	6.3	55.9	17	5.6	42.7	15	9.0	58.6	14
59-60	4.7	42.7	16	6.0	55.6	17	4.2	44.8	15	9.1	60.3	14
61-62	4.6	35.8	16	4.0	57.0	17	5.6	33.7	15	6.9	50.7	14

Nuc: Nucleated Cells; Leuk: Leukocytes.

APPENDIX D
BODY WEIGHTS OF MICROTUS (EXPERIMENT 2)

Body Weight

Body weights for the four species that were measured in Experiment 2 are located in the following table (Appendix D). The body weights were not analyzed statistically because analyses were conducted on body weights in Experiment 1. The data are presented for complete descriptive purposes.

Male meadow voles and montane voles showed large increases in body weight across the ten weeks of study (changes resulted in 57.0% and 76.7% increases of week 4 values respectively); increases in body weight for male pine voles and prairie voles were less (40.6% and 39.7% respectively). Percentage increases in body weight for females of all species were more similar in value (36.0%, 25.3%, 35.2%, and 36.6% for pine voles, prairie voles, meadow voles, and montane voles respectively).

Patterns for gain in body weight for females generally mirrored those of males, except the degree of weight gain across the study was not as great and there was less variation among species. Pine voles were clearly the lightest species in the study.

Appendix D. Mean Body Weights of *Microtus* (in g) During Olfactory Preference Study (Experiment 2).

	Species			
	Pine	Prairie	Meadow	Montane
		Males		
Week Number	(N=15)	(N=14)	(N=18)	(N=16)
Week 4	14.53 (0.63)	25.42 (0.93)	27.73 (0.77)	23.09 (0.88)
Week 7	19.24 (0.54)	31.55 (1.61)	37.77 (1.37)	35.16 (1.34)
Week 10	20.43 (0.55)	35.52 (1.61)	43.62 (1.53)	40.80 (2.11)
		Females		
Week Number	(N=15)	(N=16)	(N=18)	(N=18)
Week 4	14.37 (0.63)	24.01 (0.93)	24.85 (0.74)	21.21 (0.59)
Week 7	18.74 (0.63)	27.81 (1.43)	30.64 (1.00)	27.07 (0.87)
Week 10	19.88 (0.66)	30.08 (1.67)	33.60 (1.10)	28.97 (1.01)

Columns represent mean values (± S.E.) for pine voles (*Microtus pinetorum*), prairie voles (*M. ochrogaster*), meadow voles (*M. pennsylvanicus*) and montane voles (*M. montanus*).

APPENDIX E
ANALYSES OF CELLS FOR VAGINAL SMEARS OF *MICROTUS* (EXPERIMENT 2)

Characteristics of Cells in Vaginal Smears

Mean percentages of cell types and corresponding analyses from the vaginal smears are summarized in Appendix E-1 for prairie voles, meadow voles, and montane voles. Data for pine voles are not included because only 1 of the 15 females became perforate within the 10 week study and was excluded from further analysis. Nonparametric tests (Kruskal-Wallis ANOVA's and Mann-Whitney *U* tests) were conducted for between-species comparisons.

The mean percentages of cell types during the first test session (week 4) varied little between species, and did not differ significantly. All species had smears dominated by leukocytes (range 51% to 69%), slightly less cornified cells (20% to 37%), and relatively few nucleated cells (range 10% to 13%). However, by week 7, species differences emerged. Meadow voles and montane voles had significantly higher percentages of cornified cells, and fewer leukocytes and nucleated cells, when compared to prairie voles during week 7; no significant differences were detected between meadow voles and montane voles. By week 10, meadow voles had significantly more cornified cells than montane voles and prairie voles, meadow voles also had significantly fewer

nucleated cells than the other species. During week 10, prairie voles displayed significantly fewer cornified cells than the other two species.

Repeated-measures analyses were conducted independently for each species (Wilcoxon matched-pairs signed-rank tests). The percentages of all cell types from prairie voles did not differ significantly across the weeks of study. Thus, significant species differences were due largely to changes in the proportions of cells in meadow voles and montane voles. Generally, the smears of these two species were characterized by increasing proportions of cornified cells with reductions in leukocytes and nucleated cells across the study (see Appendix for additional post-hoc comparisons).

Appendix E-1. Percentages of Cells in Vaginal Smears of *Microtus* (Experiment 2).

	Species			Kruskal-Wallis
	Prairie	Meadow	Montane	ANOVA
Week 4	(N = 8)	(N = 10)	(N = 8)	H(2, N = 26)
Cornified	20.24	37.33^{x}	23.15^{x}	1.95^{NS}
Nucleated	10.25	11.58^{x}	13.13	0.18^{NS}
Leukocytic	69.51	51.09^{x}	63.72^{x}	2.64^{NS}
Week 7	(N = 13)	(N = 15)	(N = 17)	H(2, N = 45)
Cornified	24.42^{a}	55.87^{by}	52.52^{by}	9.66^{**}
Nucleated	14.99^{a}	5.56^{bxy}	9.37^{b}	8.34^{*}
Leukocytic	60.59^{a}	38.56^{bxy}	38.11^{by}	7.81^{*}
Week 10	(N = 14)	(N = 15)	(N = 18)	H(2,N = 47)
Cornified	27.40^{a}	67.91^{bz}	55.62^{cy}	18.35^{***}
Nucleated	9.65^{a}	3.40^{by}	8.64^{a}	12.27^{**}
Leukocytic	62.95^{a}	28.69^{bxz}	35.90^{by}	15.98^{***}

Columns represent mean percentage values for prairie voles (*M. ochrogaster*), meadow voles (*M. pennsylvanicus*) and montane voles (*M. montanus*).

Superscript letters (a,b,c) indicate results of post-hoc Mann-Whitney *U* comparisons among species and are read across a given row. Means with different letters differ significantly ($p < 0.05$). The letters (x,y,z) indicate post-hoc comparisons via Wilcoxon matched-pairs signed-ranks tests and are read down a column for each cell type.

$^{*} = p < 0.05$; $^{**} = p < 0.01$; $^{***} = p < 0.001$.

BIOGRAPHICAL SKETCH

Allen Lee Salo was born in Minot, North Dakota, on January 27, 1963. He and his family moved soon to Manistique, Michigan, in the Upper Peninsula where he grew to enjoy the outdoors along the shores of Lake Michigan. He moved to Gwinn, Michigan, in 1978 where he graduated from high school in 1981. He attended Northern Michigan University and graduated with the Bachelor of Arts degree in 1985. His graduate study began in Omaha, Nebraska, at the University of Nebraska at Omaha where he received the Master of Arts degree in 1987. His graduate studies for the doctoral degree continued at the University of Florida in the Fall of 1987. He accepted a postdoctoral position at the Medical University of South Carolina in Charleston.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Donald A. Dewsbury, Chairman
Professor of Psychology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the Department of Psychology in the College of Liberal Arts and Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1992

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Dean, Graduate School